PB 211 074

AICE-AIR-72-15

# AICE* SURVEY OF USSR AIR POLLUTION LITERATURE

## Volume XV

# A THIRD COMPILATION OF TECHNICAL REPORTS ON THE BIOLOGICAL EFFECTS AND THE PUBLIC HEALTH ASPECTS OF ATMOSPHERIC POLLUTANTS

Edited By

M. Y. Nuttonson

The material presented here is part of a survey of
USSR literature on air pollution
conducted by the Air Pollution Section
AMERICAN INSTITUTE OF CROP ECOLOGY

This survey is being conducted under GRANT R 800878
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OFFICE OF AIR PROGRAMS
of the
U.S. ENVIRONMENTAL PROTECTION AGENCY

*AMERICAN INSTITUTE OF CROP ECOLOGY
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Aromatic hydrocarbons
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Thiophenes

Benzene
Ketones
Phenols
Xylenes
Toluene

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# PREFACE

The present volume constitutes the third* compilation of the USSR technical reports on a number of investigations of the biological effects of specific air pollutants. These investigations have been conducted at various public health institutes and in departments of public health of some of the universities of that country.

Industrial emissions of various air pollutants and their adverse effect on human health have stimulated investigations of their biological effects. The great strides in the development of industrial chemistry in the USSR have called attention to the toxicological significance of the new chemical air pollutants and made it imperative to conduct investigations as to the biological effects of these chemical air pollutants. It also suggested the need to conduct investigations dealing with the public health implications of these pollutants.

The material included in this volume deals with the biological effects of low concentrations of ambient chemical toxic substances such as

(1) a number of chemical compounds used in the production of new herbicides,

(2) a number of aromatic hydrocarbons of industrial importance,

(3) those emitted by chemical and oil-chemical plants,

(4) a number of chemicals used in the production of plastics, dyestuffs, antioxidants, pharmaceutical preparations, insecticides, etc.

The results of the above studies provide in the USSR a basis for the establishment of a series of new maximum permissible concentrations for new toxic substances in the atmospheric air and constitute the scientific criteria for assessing the degree of pollution of the air medium. They also form the foundation for a number of ameliorative sanitation measures to be undertaken.

Some background information on the distribution of the Soviet industry's production machine may be of interest in connection with that country's present and potential pollution problems and investigations. The planned distribution of production in the Soviet Union favors effective exploitation of the natural resources of the USSR, especially in its eastern areas where enormous natural resources are concentrated, and has led to the creation of large industrial centers and complexes of heavy industry in many of the country's economic areas (see page ix). The many diverse climatic conditions of the country and its major economic areas as well as the geographical distribution of the Soviet Union's principal industrial and mining centers and of its principal electric power stations and power systems can be seen from the various maps presented as background material in this volume.

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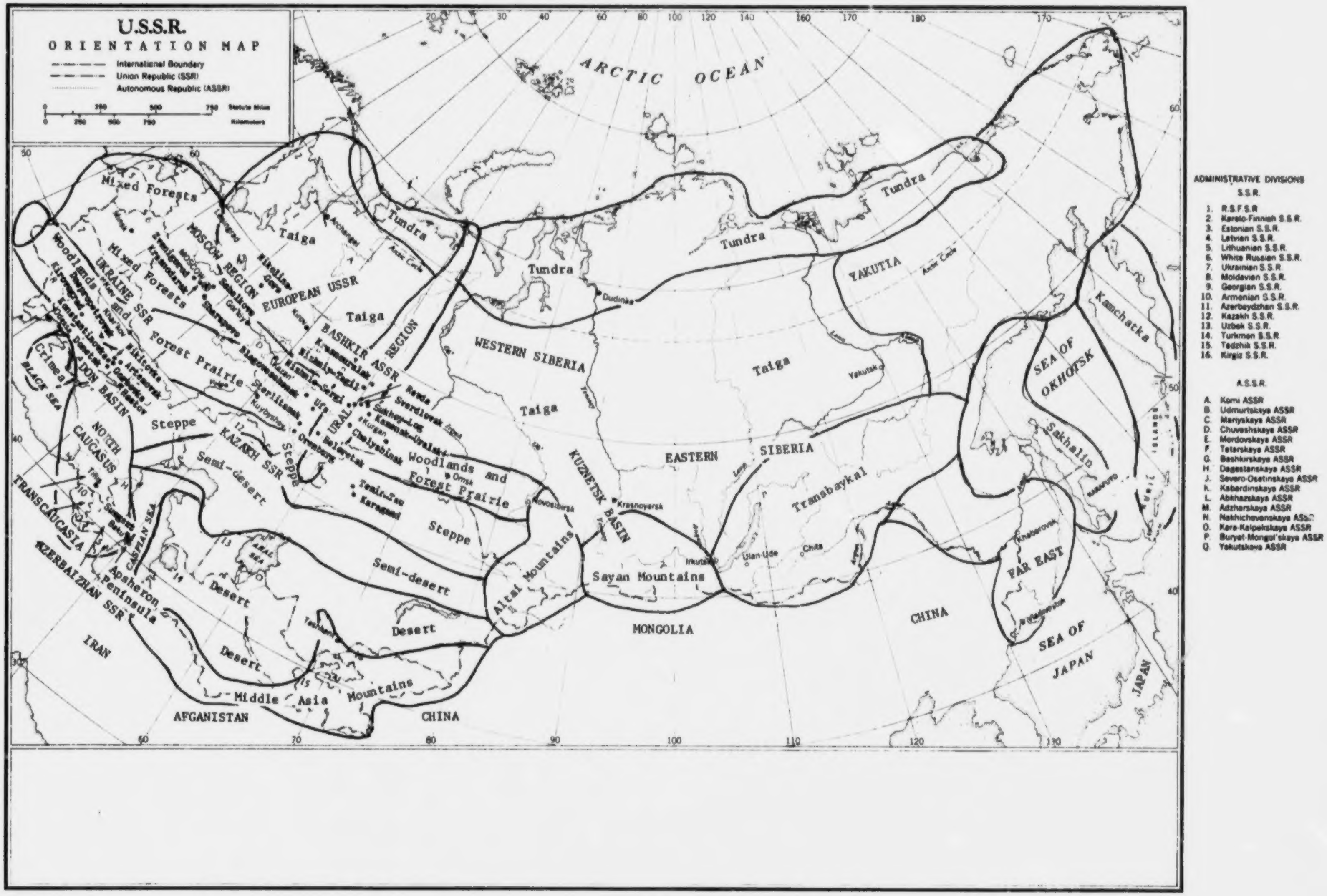
* The first compilation of this nature has been published in Volume VIII and the second, in Volume XI of the AICE Survey of USSR Air Pollution Literature.

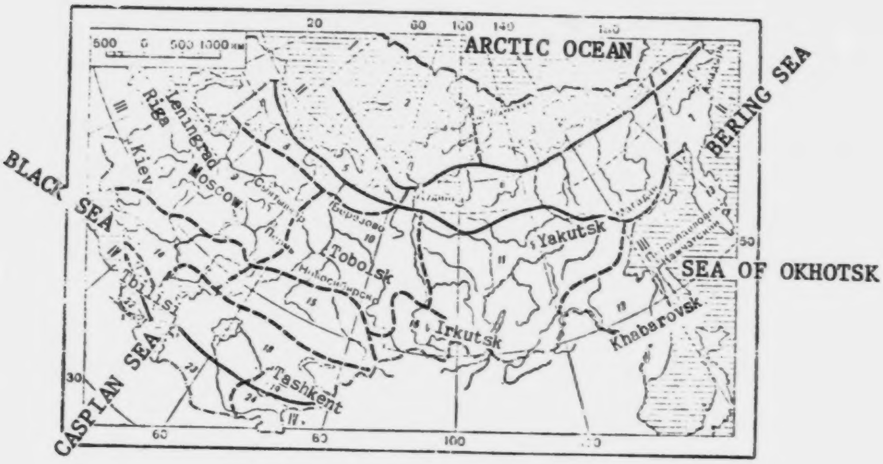
It is hoped that the papers selected for presentation in this volume will be conducive to a better appreciation of some of the air pollution investigations conducted in the USSR. As the editor of this volume I wish to thank my co-workers in the Air Pollution Section of the Institute for their valuable assistance.

M. Y. Nuttonson

July 1972


U.S.S.R.
ORIENTATION MAP
International Boundary
Union Republic (SSR)
Autonomous Republic (ASSR)
0 250 500 750 Statute Miles
0 250 500 750 Kilometers
ARCTIC OCEAN
Mixed Forests
Taiga
Tundra
MOSCOW REGION
EUROPEAN USSR
BASHKIR ASSR
REGION
URAL
UKRAINE SSR
Woodlands and Forest Prairie
Steppe
Crimea
BLACK SEA
DON BASIN
NORTH CAUCASUS
TRANSCAUCASIA
AZERBAIZHAN SSR
Apsheron Peninsula
CASPIAN SEA
KAZAKH SSR
Semi-desert
Desert
ARAL SEA
Middle Asia Mountains
AFGANISTAN
IRAN
CHINA
Woodlands and Forest Prairie
WESTERN SIBERIA
KUZNETSK BASIN
Altai Mountains
Sayan Mountains
MONGOLIA
EASTERN SIBERIA
Transbaykal
YAKUTIA
Kamchatka
SEA OF OKHOTSK
Sakhalin
FAR EAST
SEA OF JAPAN
JAPAN
Arctic Circle
Archangel
Dudinka
Krasnoyarsk
Novosibirsk
Omsk
Kurgan
Chelyabinsk
Sverdlovsk
Ufa
Orenburg
Beloretsk
Sterlitamak
Kuybyshev
Kazan'
Gor'kiy
Kirov
Temir-Tau
Karaganda
Irkutsk
Ulan-Ude
Chita
Yakutsk
Khabarovsk
Vladivostok
Baku
Sumgait
Tiflis
Kiev
Khar'kov
Dnepropetrovsk
Kirovograd
Donetsk
Rostov
Volga
Lena
Yenisey
ADMINISTRATIVE DIVISIONS
S.S.R.
1. R.S.F.S.R
2. Karelo-Finnish S.S.R.
3. Estonian S.S.R.
4. Latvian S.S.R.
5. Lithuanian S.S.R.
6. White Russian S.S.R.
7. Ukrainian S.S.R.
8. Moldavian S.S.R.
9. Georgian S.S.R.
10. Armenian S.S.R.
11. Azerbaydzhan S.S.R.
12. Kazakh S.S.R.
13. Uzbek S.S.R.
14. Turkmen S.S.R
15. Tadzhik S.S.R.
16. Kirgiz S.S.R.
A.S.S.R.
A. Komi ASSR
B. Udmurtskaya ASSR
C. Mariyskaya ASSR
D. Chuvashskaya ASSR
E. Mordovskaya ASSR
F. Tatarskaya ASSR
G. Bashkirskaya ASSR
H. Dagestanskaya ASSR
J. Severo-Osetinskaya ASSR
K. Kabardinskaya ASSR
L. Abkhazskaya ASSR
M. Adzharskaya ASSR
N. Nakhichevanskaya ASSR
O. Kara-Kalpakskaya ASSR
P. Buryat-Mongol'skaya ASSR
Q. Yakutskaya ASSR

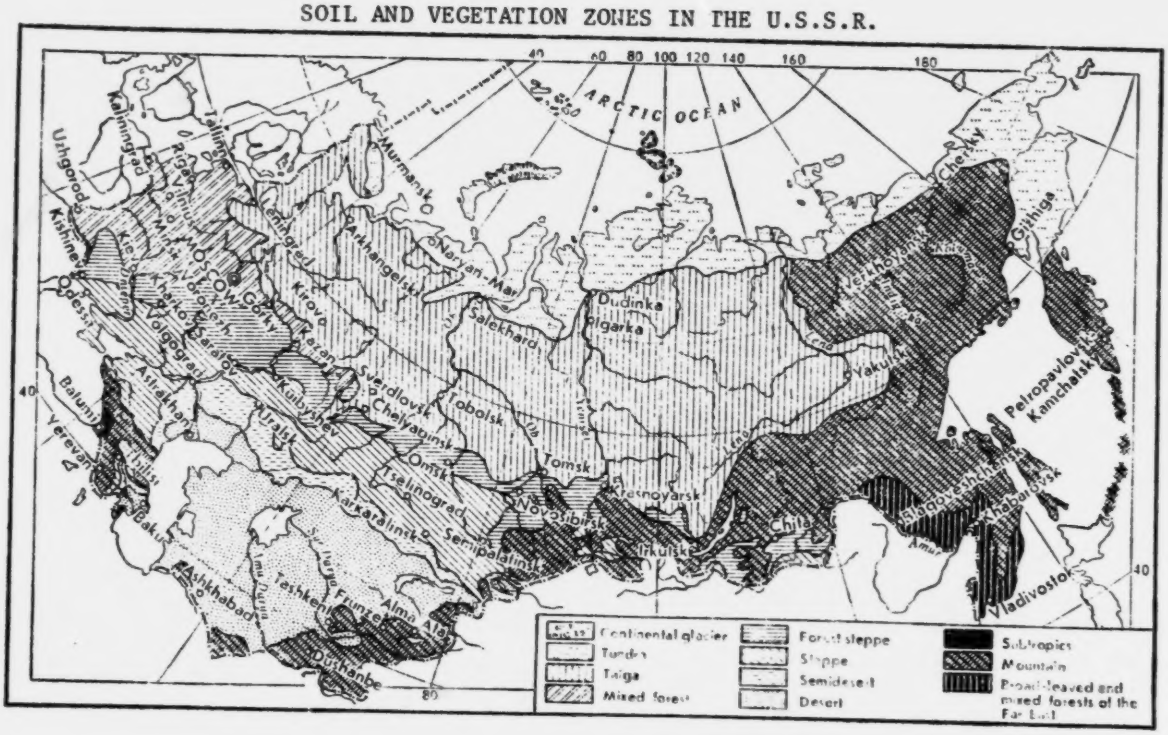
## CLIMATIC ZONES AND REGIONS* OF THE USSR


ARCTIC OCEAN
BERING SEA
BLACK SEA
SEA OF OKHOTSK
CASPIAN SEA
Riga
Leningrad
Kiev
Moscow
Tbilisi
Tobolsk
Yakutsk
Irkutsk
Khabarovsk
Tashkent
600 0 500 1000 km
20
60
100
140
180
60
80
100
30
50

Zones: I-arctic, II-subarctic, III-temperate, IV-subtropical
Regions: 1-polar, 2-Atlantic, 3-East Siberian, 4-Pacific, 5-Atlantic, 6-Siberian, 7-Pacific, 8-Atlantic-arctic, 9-Atlantic-continental forests, 10-continental forests West Siberian, 11-continental forests East Siberian, 12-monsoon forests, 13-Pacific forests, 14-Atlantic-continental steppe, 15-continental steppe West Siberian, 16-mountainous Altay and Sayan, 17-mountainous Northern Caucasus, 18-continental desert Central Asian, 19-mountainous Tyan-Shan, 20-western Transcaucasian, 21-eastern Transcaucasian, 22-mountainous Transcaucasian highlands, 23-desert south-Turanian, 24-mountainous Pamir-Alay

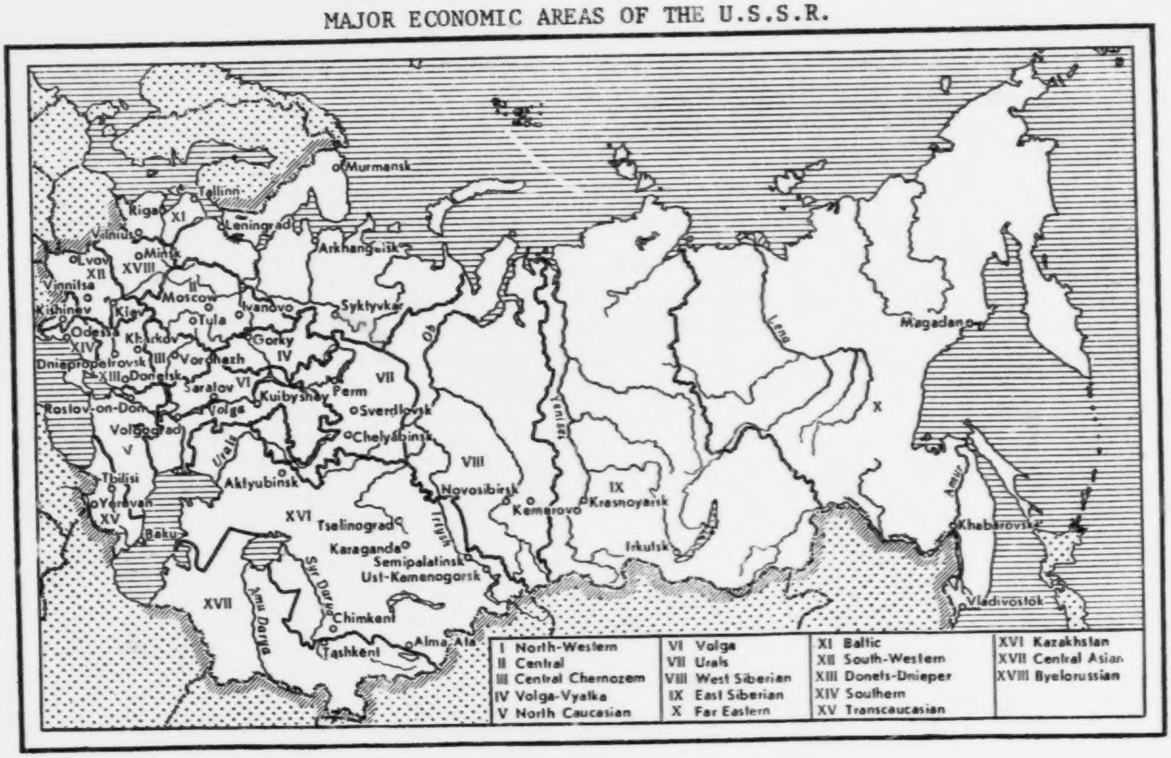
(After B. P. Alisov, "Climate of The USSR", Moscow 1956)

## SOIL AND VEGETATION ZONES IN THE U.S.S.R.


ARCTIC OCEAN
Murmansk
Arkhangelsk
Naryan-Mar
Salekhard
Dudinka
Igarka
Yakutsk
Verkhoyansk
Chersky
Gizhiga
Petropavlovsk-Kamchatsky
Kaliningrad
Uzhgorod
Riga
Tallinn
Leningrad
Minsk
Moscow
Gorky
Kirov
Kazan
Kishinev
Kiev
Odessa
Kharkov
Volgograd
Saratov
Kuibyshev
Sverdlovsk
Chelyabinsk
Tobolsk
Omsk
Tomsk
Krasnoyarsk
Novosibirsk
Irkutsk
Chita
Blagoveshchensk
Khabarovsk
Vladivostok
Amur
Astrakhan
Uralsk
Tselinograd
Karkaralinsk
Semipalatinsk
Batumi
Yerevan
Tbilisi
Baku
Ashkhabad
Tashkent
Frunze
Alma-Ata
Dushanbe
Amu Darya
Syr Darya
Ob
Lena
Yenisei
40
60
80
100
120
140
160
180
40
40
80
Continental glacier
Tundra
Taiga
Mixed forest
Forest steppe
Steppe
Semidesert
Desert
Subtropics
Mountain
Broad-leaved and mixed forests of the Far East

(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

## MAJOR ECONOMIC AREAS OF THE U.S.S.R.


Murmansk
Tallinn
Riga
Leningrad
Arkhangelsk
Vilnius
Minsk
Lvov
Vinnitsa
Kishinev
Kiev
Moscow
Tula
Ivanovo
Syktyvkar
Odessa
Kharkov
Gorky
Voronezh
Dniepropetrovsk
Donetsk
Saratov
Kuibyshev
Perm
Rostov-on-Don
Volgograd
Volga
Sverdlovsk
Chelyabinsk
Urals
Tbilisi
Yerevan
Baku
Aktyubinsk
Novosibirsk
Kemerovo
Krasnoyarsk
Irkutsk
Tselinograd
Karaganda
Semipalatinsk
Ust-Kamenogorsk
Chimkent
Tashkent
Alma-Ata
Syr Darya
Amu Darya
Irtysh
Ob
Yenisei
Lena
Amur
Magadan
Khabarovsk
Vladivostok
I North-Western
II Central
III Central Chernozem
IV Volga-Vyatka
V North Caucasian
VI Volga
VII Urals
VIII West Siberian
IX East Siberian
X Far Eastern
XI Baltic
XII South-Western
XIII Donets-Dnieper
XIV Southern
XV Transcaucasian
XVI Kazakhstan
XVII Central Asian
XVIII Byelorussian

## PLANNED DISTRIBUTION OF INDUSTRIAL PRODUCTION IN ORDER TO BRING IT CLOSER TO RAW MATERIAL AND FUEL SOURCES

An example of the planned distribution of industrial production in the USSR is the creation of large industrial centers and complexes of heavy industry in many of the country's economic areas: the North-West (Kirovsk, Kandalaksha, Vorkuta), the Urals (Magnitogorsk, Chelyabinsk, Nizhny Tagil), Western and Eastern Siberia (Novosibirsk, Novokuznetsk, Kemerovo, Krasnoyarsk, Irkutsk, Bratsk), Kazakhstan (Karaganda, Rudny, Balkhash, Dzhezkazgan).

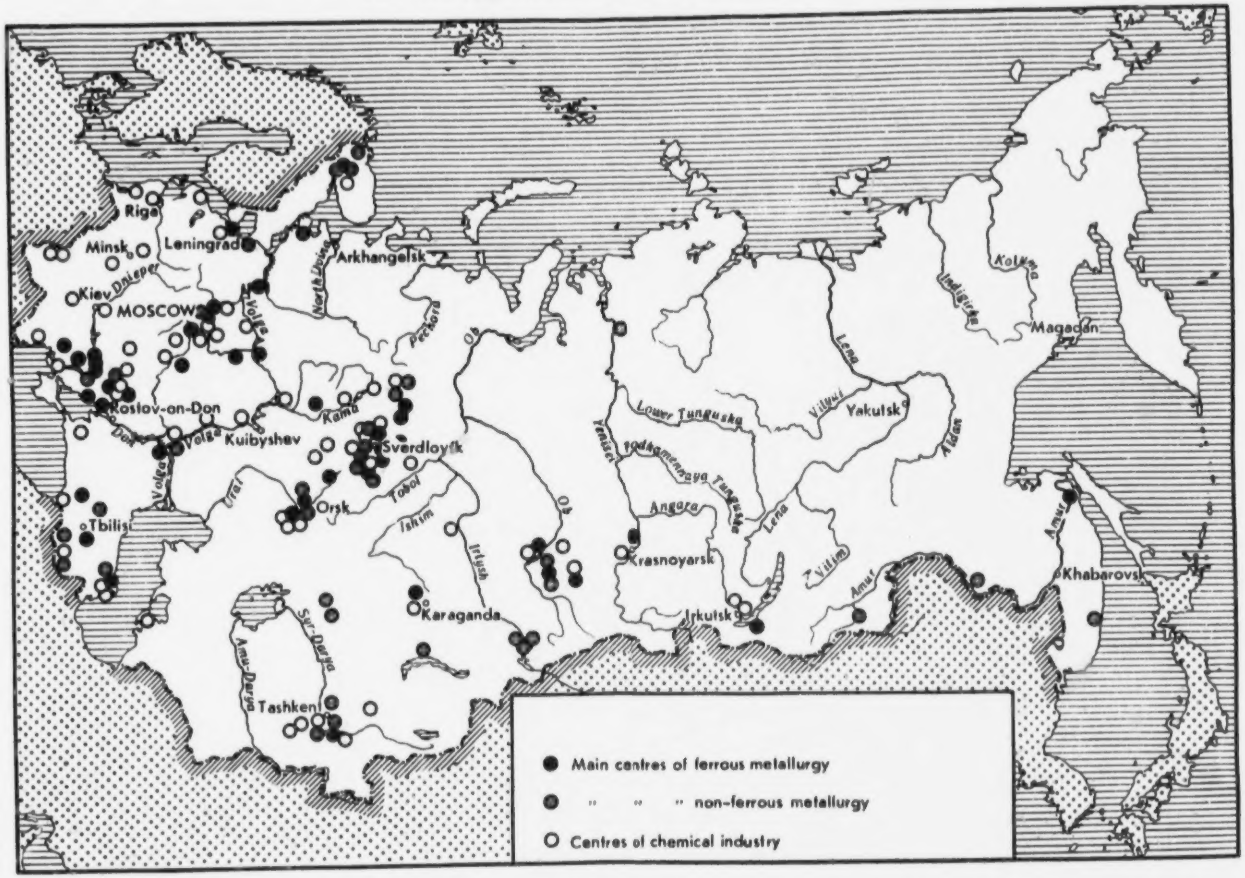
Large industrial systems are being created - Kustanai, Pavlodar-Ekibastuz, Achinsk-Krasnoyarsk, Bratsk-Taishet and a number of others. Ferrous and non-ferrous metallurgy, pulp and paper, hydrolysis and saw-milling industries are being established in the Bratsk-Taishet industrial system. The Achinsk-Krasnoyarsk industrial system is becoming one of the largest centers of aluminum and chemical industries, and production of ferrous metals, cellulose, paper, and oil products.

Construction of the third metallurgical base has been launched in Siberia, and a new base of ferrous metallurgy, using the enormous local iron and coal resources, has been created in Kazakhstan. A high-capacity power system is being organized in the same areas. Non-ferrous metallurgy is being further developed in Kazakhstan, Central Asia and in Transbaikal areas. The pulp and paper, as well as the timber, industries are being developed at a fast rate in the forest areas of Siberia and the Far East.

Ferrous metallurgy is also developing in the European part of the country by utilizing the enormous iron ore resources of the Kursk Magnetic Anomaly and the Ukrainian deposits. Large new production systems are under construction in the North-West, along the Volga, in the Northern Caucasus and the Ukraine.

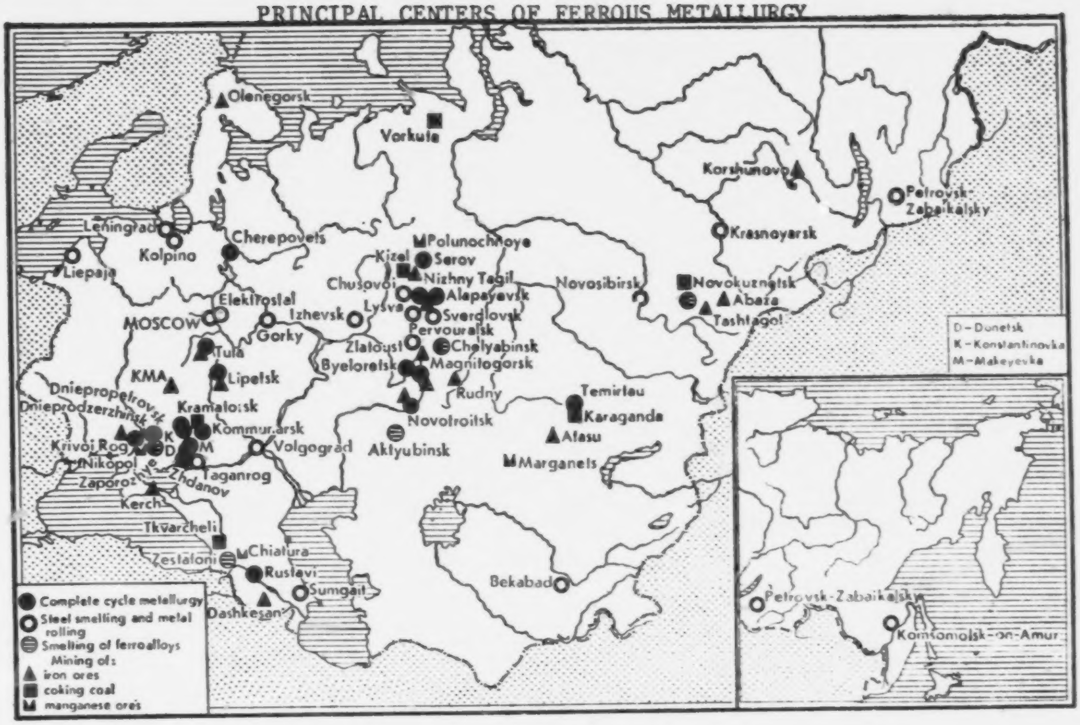
(After A. Lavrishchev, "Economic Geography
of the U.S.S.R.", Moscow 1969)

## THE MAJOR INDUSTRIAL CENTERS OF THE USSR

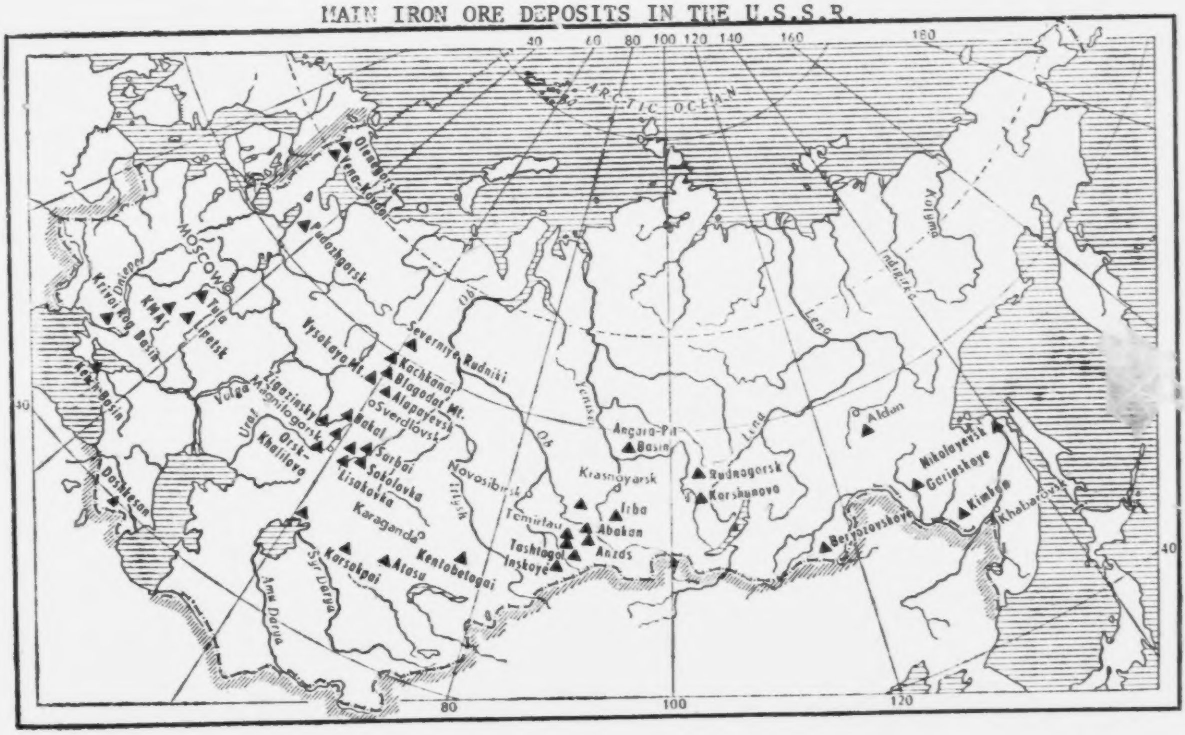

Riga
Minsk
Leningrad
Arkhangelsk
Kiev
Dnieper
MOSCOW
Volga
North Dvina
Pechora
Ob
Kama
Rostov-on-Don
Don
Volga
Kuibyshev
Sverdlovsk
Ural
Orsk
Tobol
Ishim
Irtysh
Tbilisi
Karaganda
Sur-Darya
Amu-Darya
Tashkent
Yenisei
Lower Tunguska
Podkamennaya Tunguska
Angara
Krasnoyarsk
Irkutsk
Lena
Vilyui
Yakutsk
Aldan
Vitim
Amur
Indigirka
Kolyma
Magadan
Khabarovsk
● Main centres of ferrous metallurgy
● " " " non-ferrous metallurgy
○ Centres of chemical industry

(After A. Efimov, "Soviet Industry", Moscow 1968)

## PRINCIPAL CENTERS OF FERROUS METALLURGY

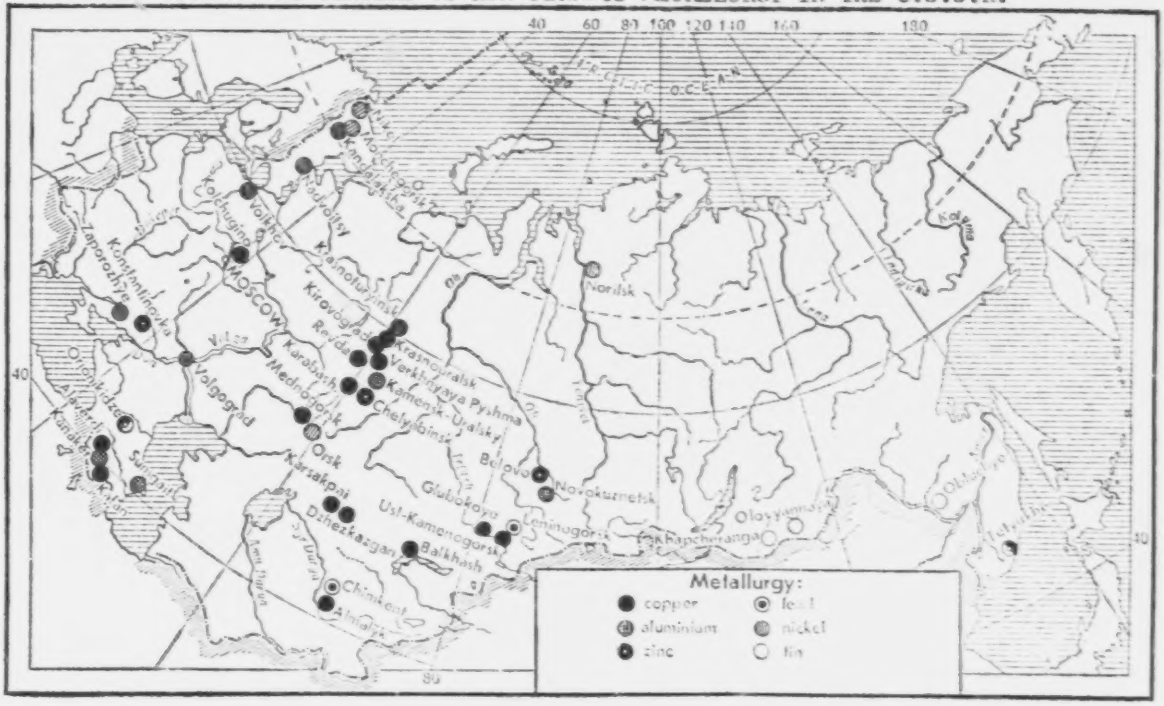

Olenegorsk
Vorkuta
Korshunovo
Petrovsk-Zabaikalsky
Krasnoyarsk
Leningrad
Kolpino
Liepaja
Cherepovets
Polunochnoye
Kizel
Serov
Nizhny Tagil
Chusovoi
Alapayevsk
Lysva
Sverdlovsk
Pervouralsk
Novosibirsk
Novokuznetsk
Abaza
Tashtagol
Elektrostal
MOSCOW
Gorky
Izhevsk
Zlatoust
Chelyabinsk
Tula
Lipetsk
Byeloretsk
Magnitogorsk
KMA
Dniepropetrovsk
Dnieprodzerzhinsk
Kramatorsk
Kommunarsk
Krivoi Rog
K
D
M
Nikopol
Zaporozhye
Zhdanov
Taganrog
Volgograd
Kerch
Rudny
Novotroitsk
Aktyubinsk
Temirtau
Karaganda
Atasu
Marganets
Tkvarcheli
Zestafoni
Chiatura
Rustavi
Sumgait
Dashkesan
Bekabad
Complete cycle metallurgy
Steel smelting and metal rolling
Smelting of ferroalloys
Mining of:
iron ores
coking coal
manganese ores
D – Donetsk
K – Konstantinovka
M – Makeyevka
Petrovsk-Zabaikalsky
Komsomolsk-on-Amur

## MAIN IRON ORE DEPOSITS IN THE U.S.S.R.

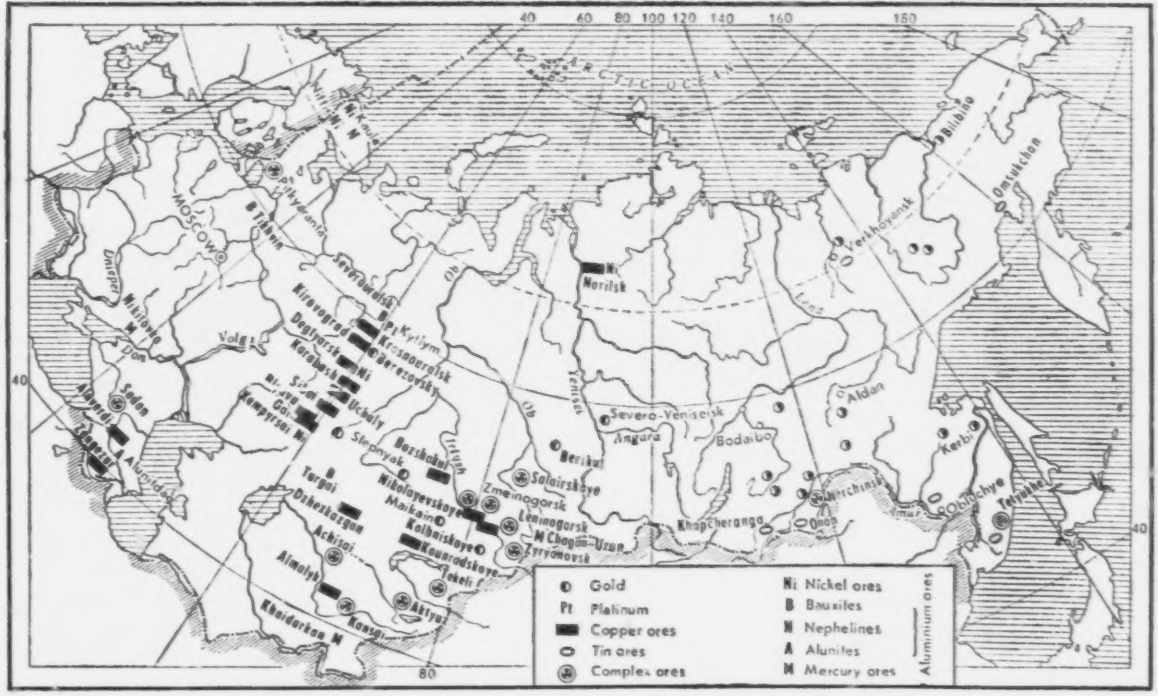

40
60
80
100
120
140
160
180
ARCTIC OCEAN
Olenegorsk
Yena-Kovdor
Pudozhgorsk
MOSCOW
Dnieper
Krivoi Rog Basin
KMA
Tula
Lipetsk
Kerch Basin
Dashkesan
Volga
Ural
Vysokaya Mt.
Severniye Rudniki
Kachkanar
Blagodat Mt.
Alapayevsk
Sverdlovsk
Zigazinsky
Magnitogorsk
Bakal
Orsk-Khalilovo
Sarbai
Sokolovka
Lisakovka
Karaganda
Kentobetogai
Atasu
Karsakpai
Syr Darya
Amu Darya
Ob
Irtysh
Novosibirsk
Temirtau
Tashtagol
Inskoye
Abakan
Anzas
Irba
Krasnoyarsk
Yenisei
Angara-Pit Basin
Rudnogorsk
Korshunovo
Lena
Aldan
Indigirka
Kolyma
Nikolayevsk
Gerinskoye
Kimkan
Khabarovsk
Beryozovskoye
40
80
100
120

(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

## PRINCIPAL CENTERS OF NON-FERROUS METALLURGY IN THE U.S.S.R.

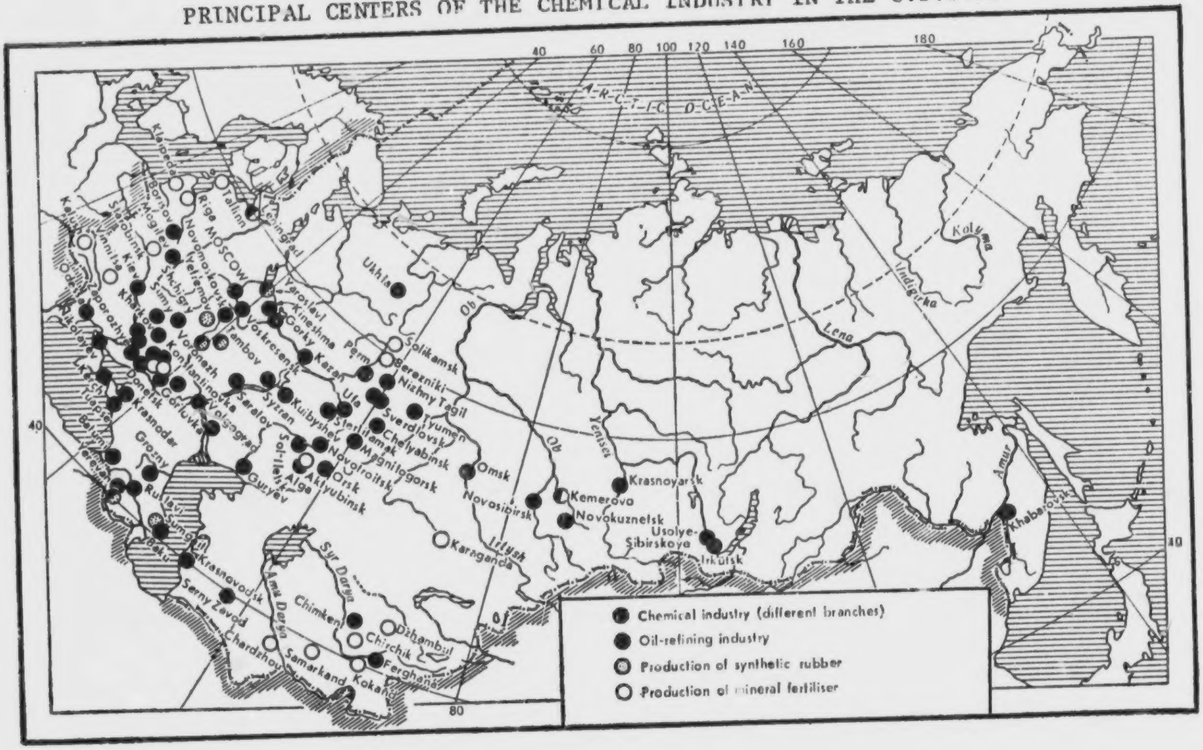

ARCTIC OCEAN
Nikel
Monchegorsk
Kandalaksha
Nadvoitsy
Volkhov
Kochugino
MOSCOW
Krasnoturyinsk
Kirovograd
Krasnouralsk
Revda
Verkhnyaya Pyshma
Karabash
Kamensk-Uralsky
Chelyabinsk
Mednogorsk
Orsk
Konstantinovka
Zaporozhye
Volgograd
Ordzhonikidze
Alaverdi
Kanaker
Kafan
Sumgait
Karsakpai
Dzhezkazgan
Balkhash
Glubokoye
Ust-Kamenogorsk
Leninogorsk
Belovo
Novokuznetsk
Norilsk
Chimkent
Almalyk
Khapcheranga
Olovyannaya
Obluchye
Tetyukhe
Metallurgy:
● copper
◉ lead
● aluminium
● nickel
● zinc
○ tin

## DISTRIBUTION OF MOST IMPORTANT DEPOSITS OF NON-FERROUS METAL ORES

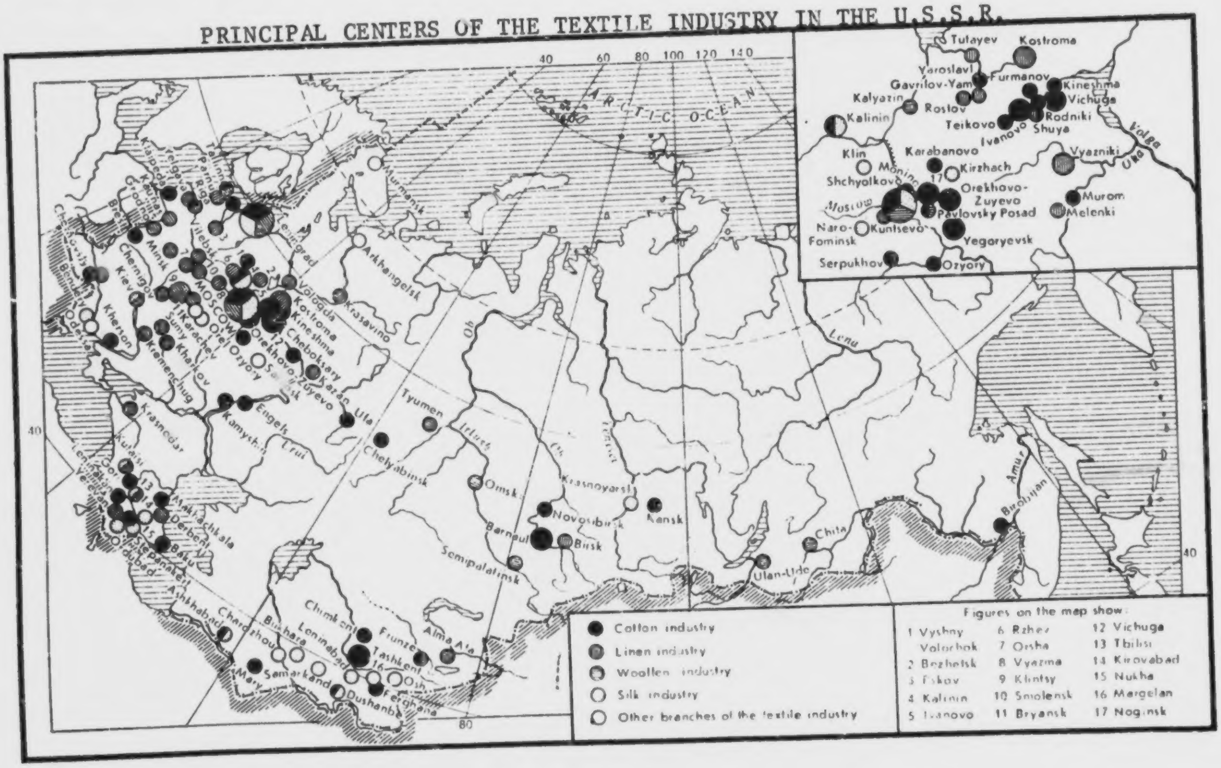

ARCTIC OCEAN
MOSCOW
Tikhvin
Severouralsk
Kytlym
Kirovograd
Krasnouralsk
Degtyarsk
Berezovsky
Karabash
Uchaly
Kempyrsai
Nikitovka
Sadon
Alaverdi
Zangezur
Alunitdag
Stepnyak
Borshokul
Turgai
Dzhezkazgan
Nikolayevskoye
Maikain
Kolbaiskoye
Kounradskoye
Tekeli
Achisai
Almalyk
Kansai
Aktyuz
Khaidarkan
Salairskoye
Zmeinogorsk
Leninogorsk
Chagan-Uzun
Zyryanovsk
Norilsk
Severo-Yeniseisk
Angara
Bodaibo
Khapcheranga
Onon
Nerchinsk
Aldan
Verkhoyansk
Bilibino
Omsukchan
Kerbi
Obluchye
Tetyukhe
Amur
● Gold
Pt Platinum
▬ Copper ores
○ Tin ores
● Complex ores
Ni Nickel ores
B Bauxites
N Nephelines
A Alunites
M Mercury ores
Aluminium ores

(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

PRINCIPAL CENTERS OF THE CHEMICAL INDUSTRY IN THE U.S.S.R.

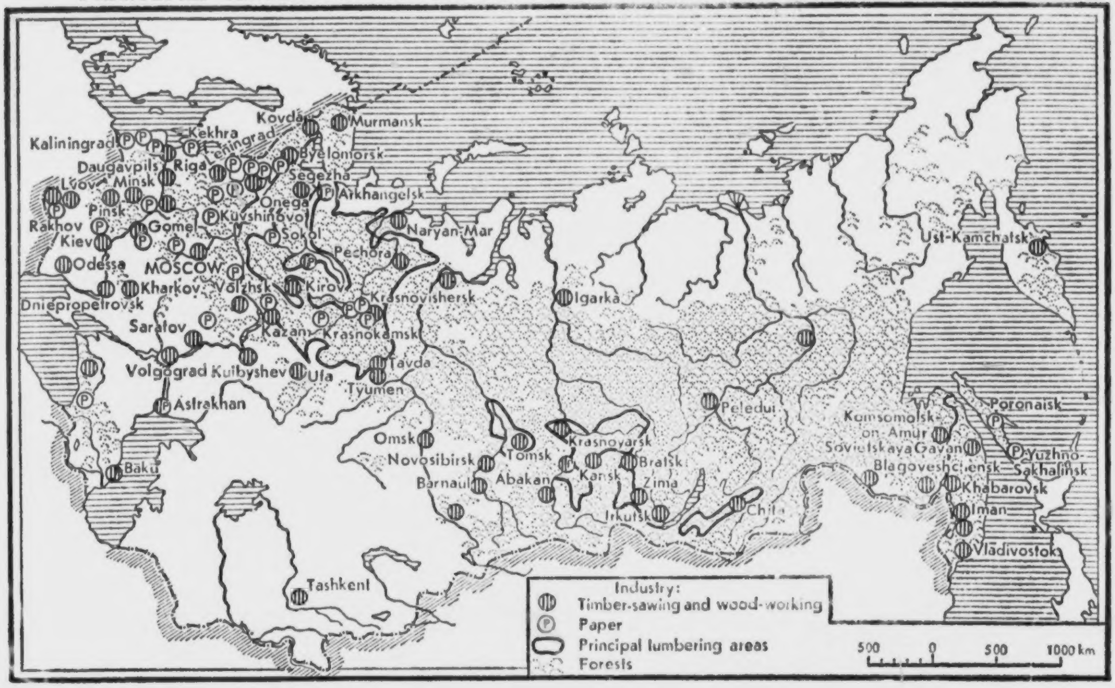

A-R-C-T-I-C O-C-E-A-N
Chemical industry (different branches)
Oil-refining industry
Production of synthetic rubber
Production of mineral fertiliser
MOSCOW
Leningrad
Yaroslavl
Kineshma
Gorky
Kazan
Perm
Solikamsk
Berezniki
Nizhny Tagil
Sverdlovsk
Tyumen
Chelyabinsk
Magnitogorsk
Ufa
Sterlitamak
Kuibyshev
Syzran
Saratov
Volgograd
Novotroitsk
Orsk
Aktyubinsk
Guryev
Grozny
Krasnodar
Baku
Krasnovodsk
Serny Zavod
Chardzhou
Samarkand
Kokand
Ferghana
Chirchik
Chimkent
Dzhambul
Karaganda
Omsk
Novosibirsk
Kemerovo
Novokuznetsk
Krasnoyarsk
Usolye-Sibirskoye
Irkutsk
Khabarovsk
Ukhta
Ob
Irtysh
Yenisei
Lena
Indigirka
Kolyma
Amur
Syr Darya
Amu Darya
40
60
80
100
120
140
160
180
80

PRINCIPAL CENTERS OF THE TEXTILE INDUSTRY IN THE U.S.S.R.

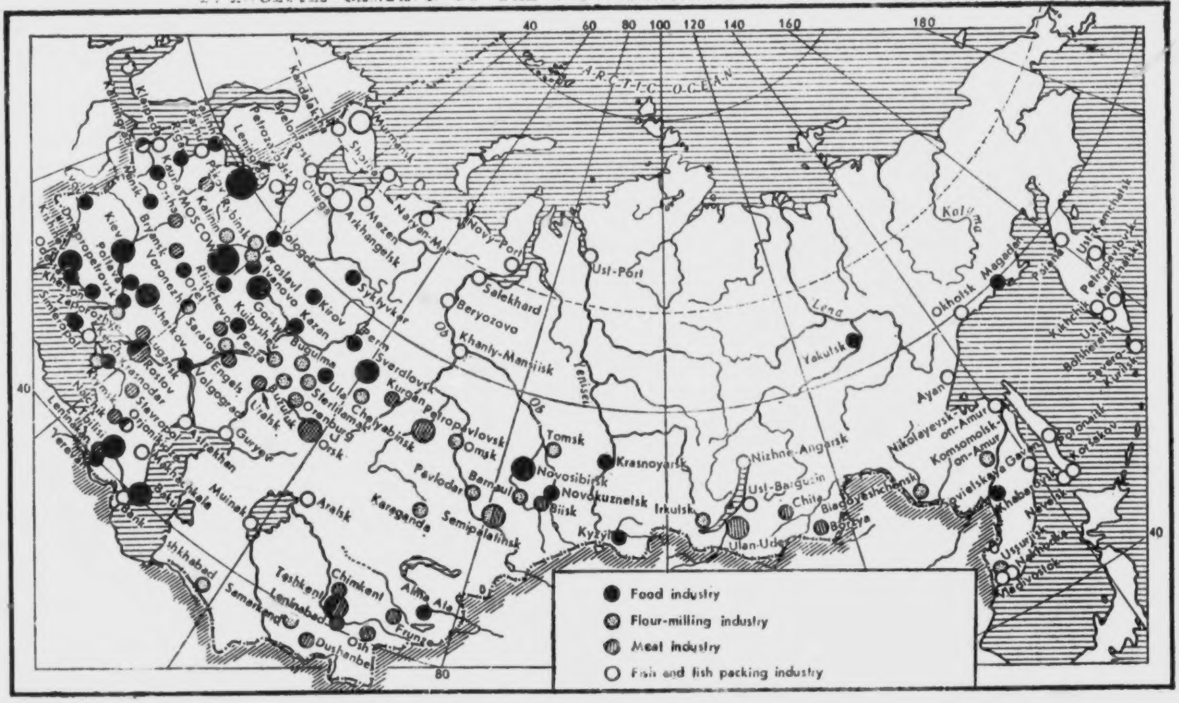

A-R-C-T-I-C O-C-E-A-N
Cotton industry
Linen industry
Woollen industry
Silk industry
Other branches of the textile industry
Figures on the map show:
1 Vyshny Volochok
2 Bezhetsk
3 Pskov
4 Kalinin
5 Ivanovo
6 Rzhev
7 Orsha
8 Vyazma
9 Klintsy
10 Smolensk
11 Bryansk
12 Vichuga
13 Tbilisi
14 Kirovabad
15 Nukha
16 Margelan
17 Noginsk
Tutayev
Kostroma
Yaroslavl
Gavrilov-Yam
Furmanov
Kineshma
Vichuga
Kalyazin
Rostov
Rodniki
Kalinin
Teikovo
Ivanovo
Shuya
Karabanovo
Klin
Kirzhach
Vyazniki
Shchyolkovo
Orekhovo-Zuyevo
Pavlovsky Posad
Murom
Melenki
Naro-Fominsk
Kuntsevo
Yegoryevsk
Serpukhov
Ozyory
Moscow
Volga
Oka
Leningrad
Arkhangelsk
Murmansk
Krasavino
Vologda
Cheboksary
Kazan
Ufa
Engels
Kamyshin
Chelyabinsk
Tyumen
Omsk
Semipalatinsk
Barnaul
Biysk
Novosibirsk
Krasnoyarsk
Kansk
Ulan-Ude
Chita
Birobidzhan
Kherson
Kiev
Minsk
Kremenchug
Krasnodar
Kutaisi
Makhachkala
Derbent
Ashkhabad
Chardzhou
Bukhara
Samarkand
Leninabad
Tashkent
Frunze
Alma-Ata
Osh
Ferghana
Dushanbe
Mary
Chimkent
Lena
Amur
Ob
Irtysh
40
60
80
100
120
140

(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

## PRINCIPAL CENTERS OF WOOD-WORKING AND PAPER INDUSTRIES IN THE U.S.S.R.

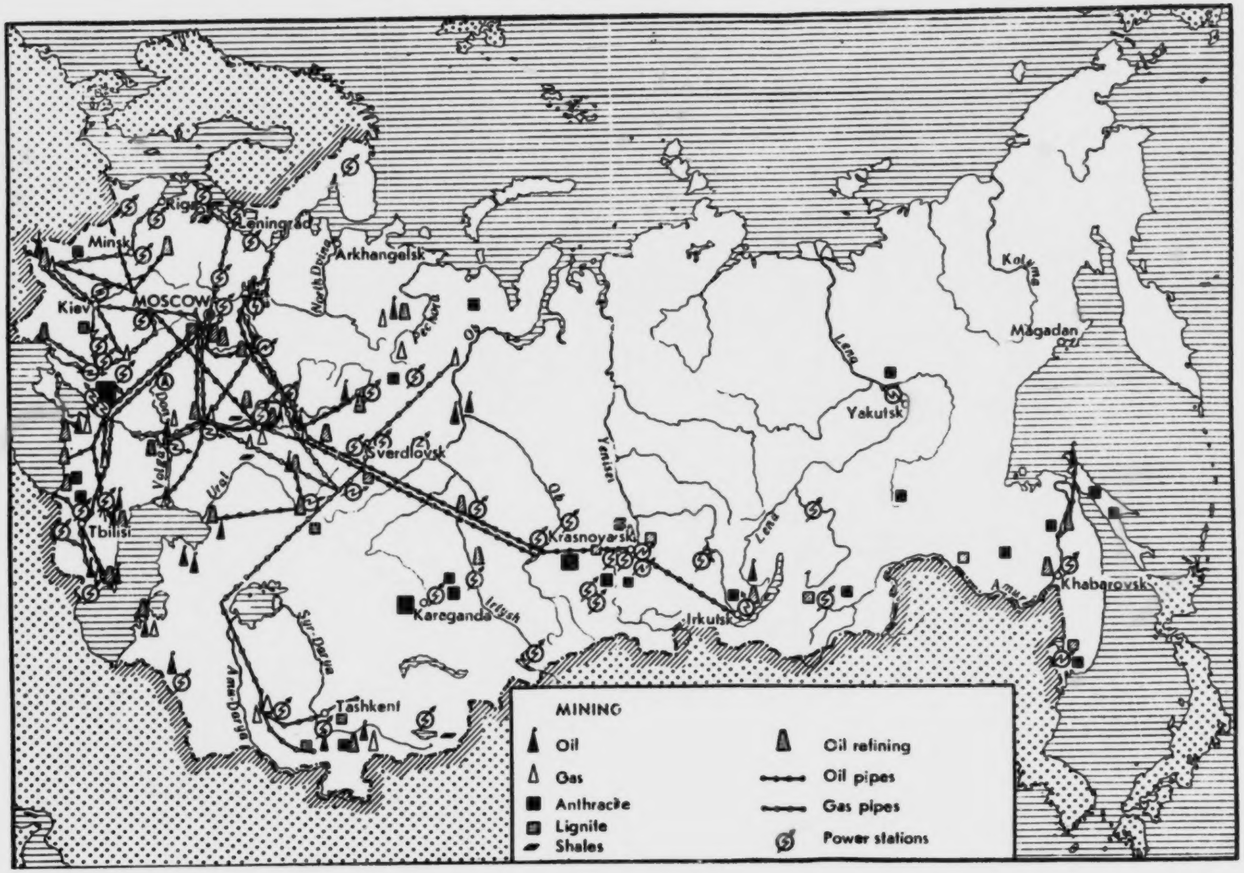

Industry:
Timber-sawing and wood-working
Paper
Principal lumbering areas
Forests
500 0 500 1000 km
Kaliningrad
Kekhra
Leningrad
Kovda
Murmansk
Daugavpils
Riga
Byelomorsk
Minsk
Lvov
Segezha
Arkhangelsk
Pinsk
Onega
Rakhov
Kuvshinovo
Gomel
Sokol
Kiev
Naryan-Mar
Pechora
Odessa
MOSCOW
Kharkov
Volzhsk
Kirov
Krasnovishersk
Dniepropetrovsk
Saratov
Kazan
Krasnokamsk
Volgograd
Kuibyshev
Ufa
Tavda
Tyumen
Astrakhan
Baku
Tashkent
Omsk
Novosibirsk
Barnaul
Tomsk
Abakan
Krasnoyarsk
Kansk
Bratsk
Zima
Irkutsk
Chita
Igarka
Peledui
Ust-Kamchatsk
Komsomolsk-on-Amur
Sovietskaya Gavan
Poronaisk
Yuzhno-Sakhalinsk
Blagoveshchensk
Khabarovsk
Iman
Vladivostok

## PRINCIPAL CENTERS OF THE FOOD INDUSTRY IN THE U.S.S.R.


Food industry
Flour-milling industry
Meat industry
Fish and fish packing industry
ARCTIC OCEAN
40
60
80
100
120
140
160
180
Murmansk
Leningrad
MOSCOW
Arkhangelsk
Mezen
Naryan-Mar
Syktyvkar
Kirov
Perm
Sverdlovsk
Chelyabinsk
Kurgan
Petropavlovsk
Omsk
Novosibirsk
Tomsk
Krasnoyarsk
Novokuznetsk
Biisk
Barnaul
Semipalatinsk
Pavlodar
Karaganda
Irkutsk
Ulan-Ude
Chita
Blagoveshchensk
Borzya
Kyzyl
Nizhne-Angarsk
Ust-Barguzin
Yakutsk
Salekhard
Beryozovo
Khanty-Mansiisk
Novy Port
Ust-Port
Okhotsk
Magadan
Ayan
Nikolayevsk-on-Amur
Komsomolsk-on-Amur
Khabarovsk
Sovietskaya Gavan
Ussuriisk
Vladivostok
Nevelsk
Korsakov
Poronaisk
Petropavlovsk-Kamchatsky
Ust-Kamchatsk
Severo-Kurilsk
Kiev
Kharkov
Rostov
Krasnodar
Volgograd
Astrakhan
Guryev
Saratov
Engels
Kuibyshev
Kazan
Ufa
Orenburg
Orsk
Sterlitamak
Gorky
Yaroslavl
Vologda
Tashkent
Chimkent
Alma-Ata
Frunze
Osh
Dushanbe
Samarkand
Ashkhabad
Aralsk
Muinak
Baku
Tbilisi
Yerevan
Odessa
Kherson
Simferopol
Kerch
Lena
Kolyma
Ob
Yenisei
80

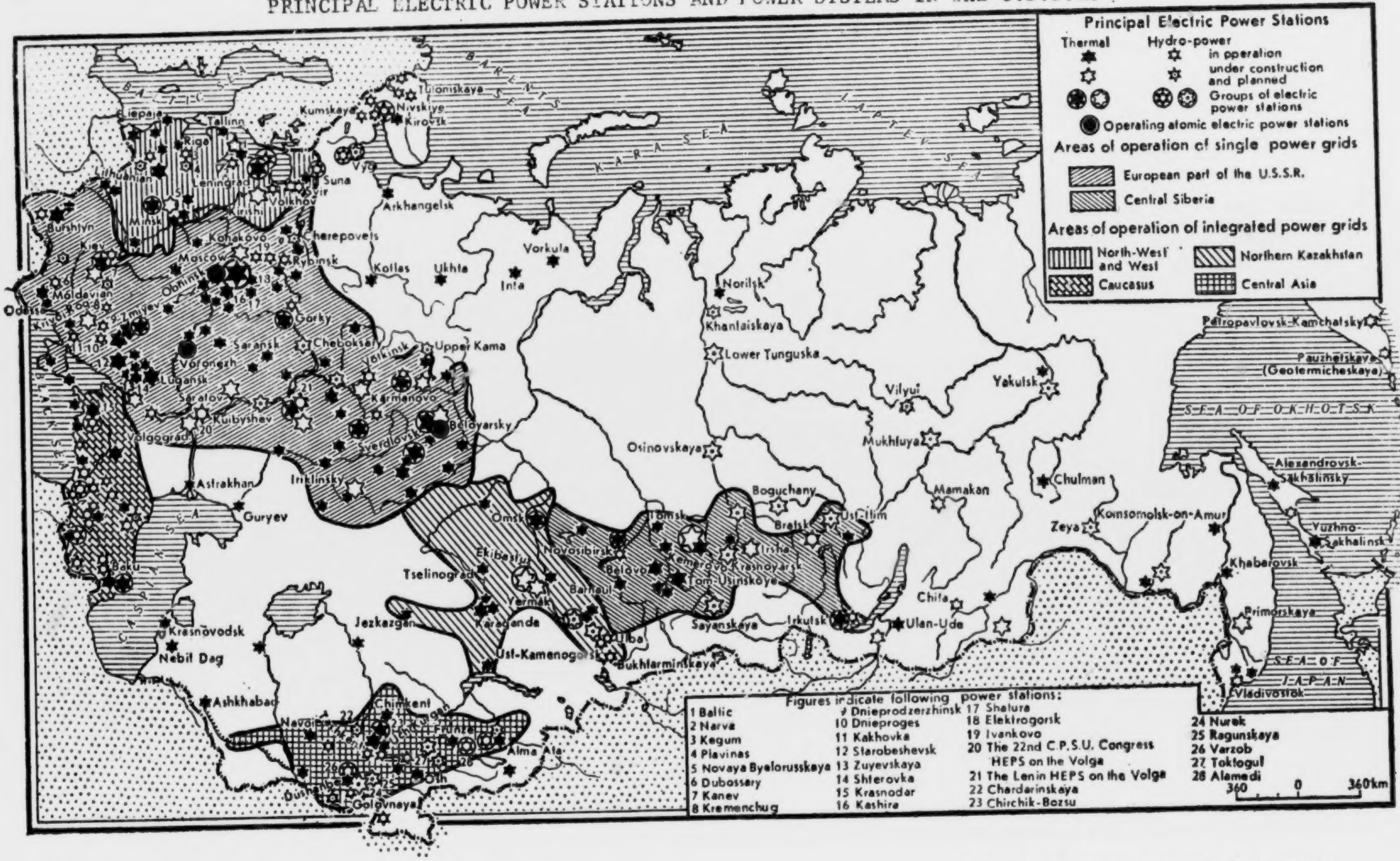
(After A. Lavrishchev, "Economic Geography
of the U.S.S.R.", Moscow 1969)

## THE MAIN MINING CENTERS OF THE USSR


Riga
Leningrad
Minsk
Arkhangelsk
North Dvina
Kiev
MOSCOW
Pechora
Ob
Don
Volga
Ural
Sverdlovsk
Tbilisi
Yenisei
Lena
Yakutsk
Kolyma
Magadan
Krasnoyarsk
Irkutsk
Karaganda
Irtysh
Syr-Darya
Amu-Darya
Tashkent
Amur
Khabarovsk
MINING
Oil
Gas
Anthracite
Lignite
Shales
Oil refining
Oil pipes
Gas pipes
Power stations

(After A. Efimov, "Soviet Industry", Moscow 1968)

## PRINCIPAL ELECTRIC POWER STATIONS AND POWER SYSTEMS IN THE U.S.S.R.


Principal Electric Power Stations
Thermal
Hydro-power
in operation
under construction and planned
Groups of electric power stations
Operating atomic electric power stations
Areas of operation of single power grids
European part of the U.S.S.R.
Central Siberia
Areas of operation of integrated power grids
North-West and West
Northern Kazakhstan
Caucasus
Central Asia
Figures indicate following power stations:
1 Baltic
2 Narva
3 Kegum
4 Plavinas
5 Novaya Byelorusskaya
6 Dubossary
7 Kanev
8 Kremenchug
9 Dnieprodzerzhinsk
10 Dnieproges
11 Kakhovka
12 Starobeshevsk
13 Zuyevskaya
14 Shterovka
15 Krasnodar
16 Kashira
17 Shatura
18 Elektrogorsk
19 Ivankovo
20 The 22nd C.P.S.U. Congress HEPS on the Volga
21 The Lenin HEPS on the Volga
22 Chardarinskaya
23 Chirchik-Bozsu
24 Nurek
25 Ragunskaya
26 Varzob
27 Toktogul
28 Alamedi
360 0 360km
BARENTS SEA
KARA SEA
LAPTEV SEA
BALTIC SEA
BLACK SEA
CASPIAN SEA
SEA OF OKHOTSK
SEA OF JAPAN
Tulomskaya
Kumskaya
Nivskiye
Kirovsk
Liepaja
Tallinn
Riga
Lithuanian
Leningrad
Suna
Volkhov
Kirishi
Minsk
Burshtyn
Kiev
Moldavian
Odessa
Krivoi Rog
Kohakovo
Moscow
Obninsk
Cherepovets
Rybinsk
Arkhangelsk
Kotlas
Ukhta
Inta
Vorkuta
Gorky
Cheboksary
Votkinsk
Upper Kama
Saransk
Voronezh
Lugansk
Saratov
Kuibyshev
Volgograd
Karmanovo
Beloyarsky
Sverdlovsk
Iriklinsky
Astrakhan
Guryev
Baku
Krasnovodsk
Nebit Dag
Ashkhabad
Navoi
Chimkent
Frunze
Alma-Ata
Osh
Dushanbe
Golovnaya
Jezkazgan
Tselinograd
Ekibastuz
Omsk
Novosibirsk
Yermak
Karaganda
Barnaul
Ust-Kamenogorsk
Ulba
Bukhtarminskaya
Belovo
Tomsk
Kemerovo
Krasnoyarsk
Tom-Usinskoye
Sayanskaya
Irkutsk
Bratsk
Ust-Ilim
Boguchany
Osinovskaya
Norilsk
Khantaiskaya
Lower Tunguska
Vilyui
Mukhtuya
Mamakan
Ulan-Ude
Chita
Yakutsk
Chulman
Zeya
Komsomolsk-on-Amur
Khabarovsk
Primorskaya
Vladivostok
Petropavlovsk-Kamchatsky
Pauzhetskaya (Geotermicheskaya)
Alexandrovsk-Sakhalinsky
Yuzhno-Sakhalinsk

(After A. Lavrishchev, "Economic Geography of the U.S.S.R.", Moscow 1969)

# MAXIMUM PERMISSIBLE CONCENTRATIONS OF NOXIOUS SUBSTANCES IN THE ATMOSPHERIC AIR OF POPULATED AREAS*

(V. A. Ryazanov)

From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 201-204, (1968).

| Pollutant | Concentration, mg/m$^3$ | |
|---|---|---|
| | Maximum single | Mean daily |
| 1 | 2 | 3 |
| 1. Nitrogen dioxide | 0,085 | 0,085 |
| 2. Nitric acid (based on $HNO_3$ molecule | 0.4 | 0.4 |
| (based on hydrogen ion) | 0,006 | 0.006 |
| 3. Acrolein | 0,30 | 0.10 |
| 4. Alpha-methylstyrene | 0.04 | 0.04 |
| 5. Alpha-naphthoquinone | 0,005 | 0.005 |
| 6. Amyl acetate | 0,10 | 0,10 |
| 7. Amylene | 1.5 | 1.5 |
| 8. Ammonia | 0,20 | 0.20 |
| 9. Aniline | 0,05 | 0.03 |
| 10. Acetaldehyde | 0.01 | — |
| 11. Acetone | 0,35 | 0.35 |
| 12. Acetophenone | 0.003 | 0,003 |
| 13. Benzene | 1.5 | 0.8 |
| 14. Gasoline (low-sulfur petroleum gasoline in terms of "C") | 5,0 | 1,5 |
| 15. Shale gasoline (in terms of "C") | 0,05 | 0.05 |
| 16. Butane | 200.0 | — |
| 17. Butyl acetate | 0.10 | 0,10 |
| 18. Butylene | 3,0 | 3.0 |
| 19. Butyl alcohol | 0.3 | — |
| 20. Butyl phosphate | 0,01 | — |
| 21. Valeric acid | 0.03 | 0,01 |
| 22. Vanadium pentoxide | — | 0,002 |

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* Approved by the Assistant Chief Public Health Physician of the USSR on 12 September 1967, No. 692-67.

| 1 | 2 | 3 |
|---|---|---|
| 23. Vinyl acetate | 0.20 | 0.20 |
| 24. Hexamethylenediamine | 0.001 | 0.001 |
| 25. Bivinyl | 3.0 | 1.0 |
| 26. Diketene | 0.007 | — |
| 27. Dimethylaniline | 0.0055 | — |
| 28. Dimethyl sulfide | 0.08 | — |
| 29. Dimethyl disulfide | 0.7 | — |
| 30. Dimethylformamide | 0,03 | 0.03 |
| 31. Dowtherm | 0.01 | 0.01 |
| 32. Dichloroethane | 3.0 | 1.0 |
| 33. 2,3-Dichloro-1,4-naphthoquinone | 0.05 | 0.05 |
| 34. Diethylamine | 0.05 | 0.05 |
| 35. Isopropylbenzene | 0.014 | 0.014 |
| 36. Isopropylbenzene hydroperoxide | 0.007 | 0.007 |
| 37. Caprolactam (vapors, aerosol) | 0.06 | 0.06 |
| 38. Caproic acid | 0.01 | 0.005 |
| 39. Malathion | 0.015 | — |
| 40. Xylene | 0.2 | 0.2 |
| 41. Maleic anhydride (vapors, aerosol) | 0,2 | 0.05 |
| 42. Manganese and its compounds (in terms of $MnO_2$) | — | 0.01 |
| 43. Butyric acid | 0.015 | 0.01 |
| 44. Mesidine | 0.003 | — |
| 45. Methanol | 1.0 | 0.5 |
| 46. Metaphos | 0.008 | — |
| 47. Metachlorophenyl isocyanate | 0,005 | 0.005 |
| 48. Methyl acrylate | 0.01 | — |
| 49. Methyl acetate | 0.07 | 0.07 |
| 50. Methyl mercaptan | $9 \cdot 10^{-6}$ | — |
| 51. Methyl methracylate | 0.1 | 0.1 |
| 52. Monomethylaniline | 0.04 | — |
| 53. Arsenic (inorganic compounds other than arsine, in terms of AS) | — | 0.003 |
| 54. Nitrobenzene | 0.008 | 0.008 |
| 55. Parachloroaniline | 0.04 | — |
| 56. Parachlorophenyl isocyanate | 0,0015 | 0.0015 |
| 57. Pentane | 100.0 | 25.0 |
| 58. Pyridine | 0.08 | 0.08 |
| 59. Propylene | 3.0 | 3,0 |
| 60. Propyl alcohol | 0.3 | — |
| 61. Nontoxic dust | 0.5 | 0.15 |
| 62. Metallic mercury | — | 0.0003 |

| 1 | 2 | 3 |
|---|---|---|
| 63. Soot (carbon black) | 0.15 | 0.05 |
| 64. Lead and its compounds (other than tetraethyl lead) in terms of Pb | — | 0,0007 |
| 65. Lead sulfide | — | 0.0017 |
| 66. Sulfuric acid (based on $H_2SO_4$ molecule) | 0.3 | 0.3 |
| (based on hydrogen ion) | 0.006 | 0.006 |
| 67. Sulfur dioxide | 0.5 | 0,05 |
| 68. Hydrogen sulfide | 0.008 | 0.008 |
| 69. Carbon disulfide | 0.03 | 0.01 |
| 70. Hydrochloric acid (based on HCl molecule) | 0.2 | 0.2 |
| (based on hydrogen ion) | 0,006 | 0.006 |
| 71. Styrene | 0.003 | 0,003 |
| 72. Thiophene | 0.6 | — |
| 73. Toluylene diisocyanate | 0.05 | 0.02 |
| 74. Toluene | 0.6 | 0.6 |
| 75. Trichloroethylene | 4.0 | 1.0 |
| 76. Carbon monoxide | 3.0 | 1.0 |
| 77. Acetic acid | 0.2 | — |
| 78. Acetic anhydride | 0.1 | — |
| 79. Phenol | 0.01 | 0.01 |
| 80. Formaldehyde | 0.035 | 0.012 |
| 81. Phosphoric anhydride | 0.15 | 0.05 |
| 82. Phthalic anhydride (vapors, aerosol) | 0.10 | |
| 83. Fluorine compounds (in terms F) | | |
| Gaseous compounds (HF, $SiF_4$) | 0.02 | 0.005 |
| Soluble inorganic fluorides (NaF, $Na_2SiF_6$) | 0.03 | 0.01 |
| Sparingly soluble inorganic fluorides ($AlF_3$, $Na_3AlF_3$, $CaF_2$) | 0.2 | 0,03 |
| In the combined presence of gaseous fluorine and fluorine salts | 0.03 | 0,01 |
| 84. Furfural | 0.05 | 0.05 |
| 85. Chlorine | 0.10 | 0,03 |
| 86. Chlorobenzene | 0.10 | 0.10 |
| 87. Chloropropene | 0.10 | 0.10 |
| 88. Hexavalent chromium (in terms of $CrO_3$) | 0.0015 | 0.0015 |
| 89. Cyclohexanol | 0.06 | 0.06 |
| 90. Cyclohexanone | 0.04 | 0.04 |
| 91. Carbon tetrachloride | 4.0 | — |
| 92. Epichlorhydrin | 0.2 | 0,2 |
| 93. Ethanol | 5.0 | 5.0 |
| 94. Ethyl acetate | 0.1 | 0.1 |

| 1 | 2 | 3 |
|---|---|---|
| 95. Ethylene | 3.0 | 3.0 |
| 96. Ethylene oxide | 0.3 | 0.03 |

REMARKS

1. In the combined presence in atmospheric air of several substances possessing a summation effect, the sum of their concentrations as calculated by the formula below (§ 2) should not exceed 1 for:

a) acetone and phenol
b) sulfur dioxide and phenol
c) sulfur dioxide and nitrogen dioxide
d) sulfur dioxide and hydrogen fluoride
e) sulfur dioxide and sulfuric acid aerosol
f) hydrogen sulfide and dowtherm
g) isopropylbenzene and isopropylbenzene hydroperoxide
h) furfural, methanol and ethanol
i) strong mineral acids (sulfuric, hydrochloric and nitric) in terms of the hydrogen ion concentration (H)
j) ethylene, propylene, butylene and amylene

should not exceed 1.3 for:

a) acetic acid and acetic anhydride

should not exceed 1.5 for:

a) acetone and acetophenone
b) benzene and acetophenone
c) phenol and acetophenone

2. Formula for the calculation:

$$X = \frac{a}{m_1} + \frac{b}{m_2} + \frac{c}{m_3} \ldots,$$

where X is the unknown total concentration:

$\frac{a}{m_1} + \frac{b}{m_2} + \frac{c}{m_3}$ — is the concentration of the substance being determined, divided by the corresponding maximum permissible concentration for isolated action.

3. In the combined presence in atmospheric air of:

a) hydrogen sulfide and carbon disulfide
b) carbon monoxide and sulfur dioxide
c) phthalic and maleic anhydrides and alpha-naphthoquinone, the maximum permissible concentrations for each of them individually are retained.

4. In the combined presence in atmospheric air of parachlorophenyl isocyanate and metachlorophenyl isocyanate, temporarily, until a method of their isolated determination is developed, the standardization should be made on the more toxic substance, i. e., parachlorophenyl isocyanate.

5. The maximum permissible concentrations of noxious substances in the atmospheric air of populated areas as formulated in December 1966 (No. 655-66), should be considered obsolete.

# SOME ASPECTS OF THE BIOLOGICAL EFFECT OF MICROCONCENTRATIONS OF TWO CHLOROISOCYANATES

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A. N. Sysin Institute of General and Communal Hygiene,
Academy of Medical Sciences of the USSR.

From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 91-107, (1968).

Chloroisocyanates (para- and metachlorophenyl isocyanates), high-molecular-weight compounds that will be used in the production of a new herbicide, parachlorophenyldimethylurea, may occur as pollutants of the atmospheric air of populated areas.*

The key element of these compounds is a benzene ring, two hydrogen atoms of which are replaced by an isocyanate group (-NCO) and a chlorine atom (-Cl): the substances differ in the position of the isocyanate group (para isomers). Parachlorophenyl isocyanate consists of white crystals with a pungent sweeetish odor. Metachlorophenyl isocyanate is a colorless liquid with a similar odor.

Data on the toxicology of these compounds are very scarce. The toxicological properties of metachlorophenyl isocyanate have not been studied at all. The effect of parachlorophenyl isocyanate on the animal body has been studied by I. N. Frolova (1962) at the level of the maximum permissible concentration for the air of industrial buildings, 0.0001 mg/l and higher. As was shown by the studies, parachlorophenyl isocyanate is a highly toxic substance. When it enters the body by inhalation, its fatal concentrations are expressed in hundredths of a milligram per liter. The zone of the toxic effect is extremely narrow. Parachlorophenyl isocyanate affects a number of the animal body functions, causing dystrophic changes in the liver, kidneys, and heart muscle.

A number of authors have indicated the ability of substances of the isocyanate group to act as allergens. Isocyanates are highly active compounds. This leads to the assumption that they react with the body proteins to form antigenic proteids (M. V. Gol'dblatt and Yu. Gol'dblatt, 1960). It should be noted that aliphatic isocyanates (hexamethylene diisocyanates) are primarily direct stimulants, whereas aromatic ones, which include para- and metachlorophenyl isocyanates, have a lesser stimulant effect and are more likely to cause a sensitization.

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*The study was made at the request of the Dzerzhinsk Branch of the State Scientific Research Institute of the Nitrogen Industry and Organic Synthesis, which initiated this project.

The toxicological properties of isocyanates have attracted the attention of researchers. Aromatic isocyanates containing a chlorine atom in the ring require a special study, since it is known that the appearance of a new chemical group or atom in the molecule causes a change in the nature of the toxic effect of a substance. The strength of the effect is considerably affected by the spatial arrangement of the substituent radicals in the molecule, i.e., by the position isomerism (A. A. Golubev and V. Ya. Rusin, 1963).

The study of the nature of the effect of chloroisocyanates as possible pollutants of atmospheric air is a very timely problem, since these compounds have not yet been introduced into industrial production.

In order to establish the degree and character of the effect of microconcentrations of para- and metachlorophenyl isocyanates, we studied the reflex responses produced by the stimulation of the human respiratory organs by these substances, and their resorptive effect on the body of animals.

The results of the experiments were evaluated by the range method (R. N. Biryukova, 1962) by considering the significance of the difference between indices obtained under the influence of different concentrations of chloroisocyanates and during inhalation of pure air (reflex effect) and the differences between the indices of experimental and control groups of animals (resorptive effect).

The content of chloroisocyanates in air was checked by using a modified variant of the method developed by A. A. Belyakov for determining the content of parachlorophenyl isocyanate in the air of industrial buildings. The method is based on alkaline hydrolysis: parachlorophenyl isocyanate is converted into parachloroaniline, the latter undergoes diazotization in a mixture containing sodium nitrite and bromide, and azo coupling is carried out with alpha-naphthol; a compound is thus formed which gives a pink color to the solution. The color intensity is measured with a photoelectrocolorimeter. The sensitivity of the method is 1 μg in the sample. Under the direction of M. V. Alekseyeva, the sensitivity of the method was increased by introducing changes into the relative proportions and volume of the reactants; in the new modification, the method permits the detection of as little as 0.05 μg of parachlorophenyl isocyanate in the sample. The procedure proved applicable to the determination of metachlorophenyl isocyanate as well (with the same sensitivity).

In the study of the reflex effect of chloroisocyanates on the human body, the thresholds of olfactory perception were determined, and the influence of microconcentrations of these substances on the light sensitivity of the eye and the electrical activity of the human brain were studied by using physiological methods employed for the standardization of atmospheric pollutants.

As is evident from Table 1, parachlorophenyl isocyanate affects the reflex responses of man in lower concentrations than metachlorophenyl isocyanate. It is noteworthy that the odor subthreshold concentration of metachlorophenyl isocyanate (0.008 mg/m$^3$) does not alter the course of the dark adaptation curve, in contrast to the first substance; the threshold concentration of 0.010 mg/m$^3$ is active. No such differences in the nature of action of the substances were observed in the electroencephalographic studies.

Table 1

Thresholds of Reflex Effect of Chloroisocyanates.

| Substance | Olfactory Perception | | Light Sensitivity of the Eye | | Electrical Activity of the Brain | |
|---|---|---|---|---|---|---|
| | Concentrations, mg/m$^3$ | | | | | |
| | Minimum Perceptible | Maximum Imperceptible | Minimum Active | Maximum Inactive | Minimum Active | Maximum Inactive |
| Parachlorophenyl isocyanate . . . | 0,015 | 0,0064 | 0,0068 | 0,0029 | 0,0029 | 0,0015 |
| Metachlorophenyl isocyanate . . . | 0,010 | 0,008 | 0,010 | 0,008 | 0,008 | 0,005 |

As has been shown by many authors, the method of electroencephalography permits a quantitative consideration of the change of the functional state of the central nervous system during the action of low concentrations of toxic substances, and is the most sensitive method for the study of the reflex effect of atmospheric pollutants on the human body. In our studies, we used the "alpha-rhythm-burst response" technique developed by A. D. Semenenko. A statistically significant depression of the alpha rhythm was caused by chloroisocyanates in concentrations that did not affect the light sensitivity of the eye. Parachlorophenyl isocyanate did not change the electrical activity of the brain in a concentration of 0.0015 mg/m$^3$, nor did metachlorophenyl isocyanate in a concentration of 0.005 mg/m$^3$.

The method is objective, and the work of the experimenter is considerably facilitated and more accurate when a biocurrent integrator is connected. However, the duration of the experiment and possibly the excessive load on the central nervous system cause fatigue in the subject in the majority of cases, and this has an adverse effect on the course of the experiment. We therefore set up experiments in accordance with a shortened variant of the "alpha-rhythm-burst response" technique proposed by Semenenko with a smaller load for the subject. The duration of the test was cut by one-half, i.e., it lasted only 9 minutes. The entire experiment as usual consists of 18 cycles, each of which lasts half a minute, not one minute as before. The cycle begins with the supply of flickering light (13 seconds); during that time, the light intensity is changed 2 to 3 times. This is followed by limbering up (about 10 seconds), which is interrupted by a sound signal; during the next 4-5 seconds the subject waits. The specified concentrations of the substance are supplied in fractions, during the flickering light.

When this technique is employed, the experiment is set up under obviously difficult conditions: the time of supply of the "gas" is shortened, and the quantitative analysis of the curve is carried out in only 10 seconds. If the effect of the substance studied can be obtained under these conditions, it is probably more reliable, which proves the sensitivity of the method. Chloroisocyanates in the same concentration caused a decrease in the amplitude of the alpha rhythm that was sufficiently extensive and lasting to be confirmed statistically. The inactive concentrations were also in agreement.

These concentrations (0.0015 mg/m$^3$ parachlorophenyl isocyanate and 0.005 mg/m$^3$ metachlorophenyl isocyanate) are proposed as the highest single maximum permissible concentrations for atmospheric air.

When the organism is exposed to different compounds, aspects of the effect that are specific and characteristic of certain substances can be noted as well as a number of nonspecific changes in the state of the organism that are of the same type for different poisons. When the effect of low and microconcentrations of atmospheric pollutants is studied, its specific features disappear, and nonspecific shifts, which are regarded as a straining of the defensive reactions of the body in response to the action of the chemical substances, assume a special importance. The mobilization of the defenses indicates that the ambient medium does not match the sanitary level.

In order to determine the toxic effect of chloroisocyanates on the animal body, a round-the-clock inhalational experiment was carried out on white rats (males) for 80 days. The resorptive effect of para- and metachlorophenyl isocyanates was studied in concentrations equal to the highest single maximum permissible concentrations (0.0015 and 0.005 mg/m$^3$ respectively) and concentrations 20 times as high (0.03 and 0.1 mg/m$^3$).

During the experiment, several indices were observed: ratio of the rheobases and chronaxies of antagonist muscles; excretion of coproporphyrin with the urine (M. I. Gusev and Yu. K. Smirnov, 1960); electrophoretic separation of the protein fractions of the blood serum (A. Ye. Gurvich, 1955) with refractometric determination of total protein; counting of the absolute number of eosinophils in peripheral blood (S. M. Bakman, 1958); determination of neutral 17-ketosteroids in the urine; amperometric titration of sulfhydryl groups in the blood serum. At the end of the exposure and of the recovery period, the ascorbic acid content was studied in the adrenal glands, kidneys, brain, and liver by titrating with 2.6-dichlorophenol indophenol; pyruvic acid was determined in the liver by the Friedemann-Haugen method, the sulfhydryl groups were determined in the liver (N. N. Pushkina, 1963), and sialic acids in the blood serum were determined by the method of Hess modified by Pushkina.

In the study of the effect of smaller concentrations (0.0015 mg/m$^3$ parachlorophenyl isocyanate, 0.005 mg/m$^3$ metachlorophenyl isocyanate), no statistically significant difference in the indices of the experimental and control groups of animals was found in any of the tests. These concentrations may be recommended as the mean daily maximum permissible ones.

Let us consider the effect of large concentrations: 0.03 mg/m$^3$ parachlorophenyl isocyanate and 0.1 mg/m$^3$ metachlorophenyl isocyanate.

Electrophoretic analysis of the blood serum proteins (Fig. 1) revealed an appreciable decrease of the albumin-globulin ratio (more marked and lasting in the presence of parachlorophenyl isocyanate). In addition, parachlorophenyl isocyanate is characterized by a statistically significant increase of the total globulins owing to an increase of the gamma globulin content and, to a lesser extent of the beta globulin fraction during the first half of the exposure. The decrease of the albumin-globulin ratio during inhalation of methylchlorophenyl isocyanate was due to the presence, on the one hand, of a tendency toward a decrease in the amount of albumin, and on the other hand, to an increase of globulins (in one case this increase was significant). In the individual globulin fractions, only the increase of alpha globulin content was found to be appreciable; during the action of parachlorophenyl isocyanate, this increase appeared in the second half of the experiment, replacing the increase of the gamma and beta globulin fractions. At the same time, a statistically significant decrease of albumins was also observed.

S. Ya. Kaplanskiy (1962) has pointed out that when the body is acted upon by harmful agents, instead of the albumins leaving the circulatory system, the latter is penetrated by alpha globulins originating in the liver, some of which have the same electrophoretic mobility as the corresponding serum globulins. Similar changes in the ratio of protein fractions of the blood serum apparently arise under the influence of metachlorophenyl isocyanate and, during the second period of exposure, parachlorophenyl isocyanate.

As far as the increase in total globulins and particularly in the gamma globulin fraction during the action of parachlorophenyl isocyanate is concerned, it is known that allergic disorders always involve changes in the protein fractions of the blood serum with a regular increase of individual globulins (V. I. Pytskiy, 1963). Globulins are highly reactive proteins that readily combine with various substances. Chemical substances of non-protein nature causing allergy usually are not complete antigens and constitute determinant groups, which acquire sensitizing properties only after combining with the body proteins. General activation of protein synthesis is a prerequisite without which there can be no manufacture of a protein that is new for an organism and characterized by a specific affinity for a given antigen. The majority of authors relate the formation of antibodies to gamma globulins, which display a rate of displacement equal to that of antibodies in an electric field. In addition, it is well known that the production of antibodies and gamma globulins is carried out by cells of the plasma series (P. F. Zdrodovskiy, 1961).

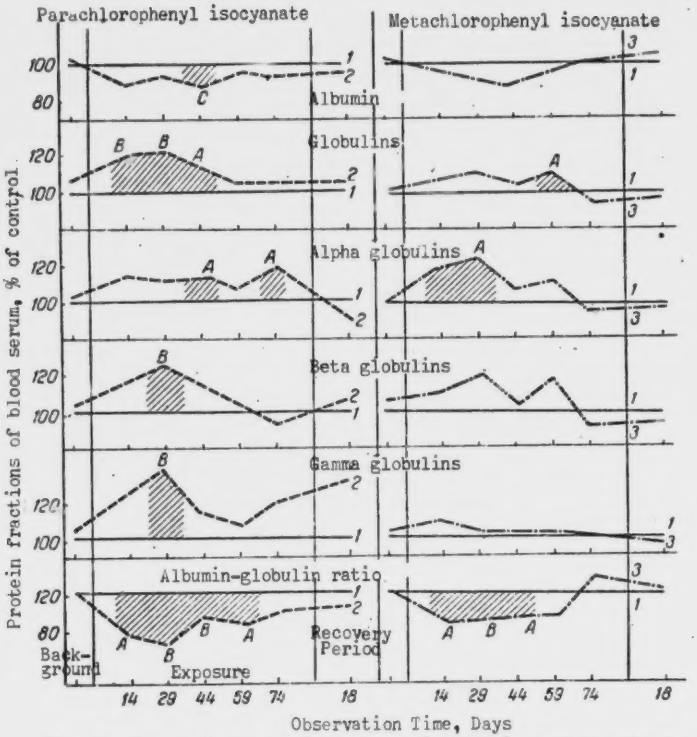

Fig. 1. Change in the ratios of protein fractions of the blood serum during the action of parachlorophenyl isocyanate (0.03 mg/m$^3$) and metachlorophenyl isocyanate (0.1 mg/m$^3$).
1 - pure air; 2 - parachlorophenyl isocyanate, 0.03 mg/m$^3$; 3 - metachlorophenyl isocyanate, 0.1 mg/m$^3$. The areas of statistically significant changes are crosshatched. Degree of significance: a - 95.0%; b - 99.9%.

Under the influence of various factors, a change in the amount of eosinophils in the blood takes place in the direction of both an increase and a decrease of their number depending on the nature of the interaction.

Considering that the number of eosinophils in the blood is a variable quantity subject to fluctuations in the course of a day (daily rhythm), the blood of rats was taken under identical and constant conditions (before feeding), preferably during the morning hours, when the number of eosinophils was the lowest.

The number of eosinophils in rats receiving metachlorophenyl isocyanate did not differ from their number in the control animals during the entire course of exposure. Parachlorophenyl isocyanate induced a statistically significant increase of the eosinophil content of the blood. Eosinophilia (the number of eosinophils was 42% higher than in animals of the control group) corresponded in time to the increase of the gamma globulin fraction of the blood serum and was absent in the second half of the exposure (Fig. 2). One of the constant symptoms of allergy is eosinophilia, observable in the blood, tissues, and mucous membranes. An increased bone marrow eosinophilopoiesis is thought to take place during sensitization. Yu. F. Valiyev (1964) indicates the simultaneous presence of a relatively distinct relation between the increase in the phagocytic capacity of eosinophiles and their content in different intracellular substances, including those formed during an allergic reaction. It is possible that eosinophils active toward these substances are transported with the blood stream toward the "shock" organs where the allergic reaction becomes localized. In the lungs and spleen of the experimental animals, accumulations of eosinophilic leucocytes were observed which were not found in the organs of the control animals.

In different experimental studies, the investigation of the functional state of the adrenal glands through observation of the excretion of neutral 17-ketosteroids with the urine is widely employed. At the start of exposure to parachlorophenyl isocyanate, the content of 17-ketosteroids in the urine of the experimental animals did not differ substantially from the background indices (see Fig. 2). Later, simultaneously with a normalization of the content of the gamma globulin fraction and a certain decrease in the total globulins and in the number of eosinophils in the blood to the level of the control, there occurred an increase in the excretion of 17-ketosteroids with the urine (the confidence factor of the changes was 99%). A change in the content of 17-ketosteroids in the urine of rats during the action of metachlorophenyl isocyanate occurred much earlier, although it was insignificant in the first half of the experiment.

An intensified excretion of 17-ketosteroids indicates an increase in the functional activity of the hypophyseal-adrenocortical system, which characterizes stress reactions to harmful factors. According to the data of F. M. Shleyfman (1961), the greatest changes in the functional state of adrenal glands are observed precisely during the period of progressive adaptation to an external irritant. A similar interpretation of the increased excretion of 17-ketosteroids may be entirely attributed to the action of metachlorophenyl isocyanate.

During the action of parachlorophenyl isocyanate, the reaction of the adrenal glands apparently has a more specific character and takes place in response to the sensitizing effect of the substance. As a result of the increased activity of the hypophyseal-adrenocortical system, there is an enhancement of the excretion of gluco-corticoids, which, because of their antiallergic effect, depress the plasmacytic reaction (P. F. Zdrodovskiy, 1961, 1964) and hence, antibody production, and depress eosinophilopoiesis. We tend to regard the increase of the total globulins in the blood serum with a predominance of the gamma globulin fraction, eosinophilia and the superseding increase of excretion of neutral 17-ketosteroids indicating an intense formation of gluco-corticoids, as indications of a possible sensitizing effect of parachlorophenyl isocyanate (0.03 mg/m$^3$). These indications disappear when the

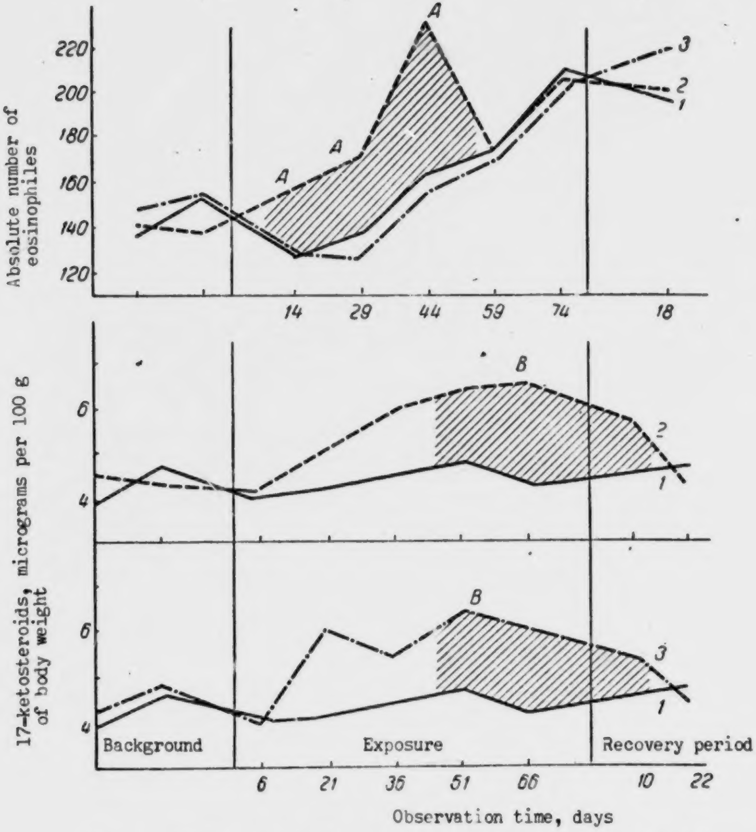

Fig. 2. Effect of chloroisocyanates on the level of eosinophiles in peripheral blood and excretion of neutral 17-ketosteroids with the urine. Notation same as in Fig. 1.

humoral medium in which the reaction between the antigen and the corresponding antibodies takes place changes in a direction unfavorable to the occurrence of sensitization processes. It should also be kept in mind that there is no single test which could completely determine the allergic nature of the effect; it becomes necessary to content oneself with only a set of positive data (B. S. Preobrazhenskiy, 1964).

We were interested in the supply of vitamins to the organism of the experimental animals: in the adrenal glands, a decrease in the content of ascorbic acid, which is involved in the oxidative transformation of steroid hormones, is a manifestation of an increase in the functional activity of the adrenals; the process of antibody formation is sensitive to a deficiency of vitamins. The content of ascorbic and pyruvic acids (the latter may be used as an indicator of the level of vitamin $B_1$ in the body) was determined in the organs of the rats. Results of the analyses are given in Table 2.

Table 2

Change of Certain Biochemical Indices During the Action of Parachlorophenyl Isocyanate and Metachlorophenyl Isocyanate.

| Biochemical Index | Control (Pure Air) | Parachlorophenyl isocyanate, 0.03 mg/m$^3$ | Metachlorophenyl isocyanate, 0.1 mg/m$^3$ |
|---|---|---|---|
| Vitamin C, mg% | | | |
| Adrenal glands . . | 525,4±17,0 | 332,2±24,7 (c) | 320,1±21,2 (c) |
| Kidneys . . . . . . | 18,8±1,4 | 10,4±1,3 (b) | 9,5±1,7 (b) |
| Brain . . . . . . . | 39,4±1,7 | 29,4±1,3 (b) | 29,2±1,9 (b) |
| Liver . . . . . | 29,4±2,9 | 21,8±1,3 (a) | 21,0±1,9 (a) |
| Liver pyruvic acid, mg % | 1,9±0,6 | 5,0±0,6 (b) | 4,2±0,7 (a) |
| Sulfhydryl groups, μmoles . . . | 0,90±0,01 | 0,53±0,11 (a) | 0,75±0,02 (c) |
| Sialic acids of Blood serum, mg % | 152,8±4,7 | 263,0±28,0 (b) | 229,6±19,3 (b) |

Remarks. 1. The table gives values of M + m. 2. Degree of significance: a - 95%; b - 99%; c - 99.9%

Chloroisocyanates caused a decrease of vitamin C level in the adrenal glands, kidneys, brain, and liver; this decrease was most pronounced in the kidneys and adrenal glands (by 45.7-36.7%). Rats have the ability to synthesize ascorbic acid in the course of intermediate metabolism in response to the influence of unfavorable factors of the ambient medium (B. A. Lavrov and B. I. Yanovskaya, 1956).

However, during a prolonged action of chloroisocyanate, the increased biosynthesis of vitamin C evidently does not compensate for the greater demand of the rat organism for this vitamin, and this results in a decrease of the content of ascorbic acid not only in the adrenal glands but also in other organs.

An accumulation of pyruvic acid was observed in the liver. A delay in the splitting of this acid can be used as an indication of hypovitaminosis $B_1$, since thiamine in the form of the pyrophosphate is a coenzyme participating in the oxidative decarboxylation of pyruvic acid. Z. S. Gershanovich and A. I. Minkina (1951) found that vitamin $B_1$ retards the oxidation of ascorbic acid. A decrease of the thiamine level as a result of the action of chloroisocyanates promotes the consumption of vitamin C in the rat organism even further.

These vitamins also share an affinity for the sulfhydryl groups of proteins. Ascorbic acid is thought to be involved in the metabolism of glutathione and other sulfhydryl groups. The presence of a certain amount of sulfhydryl groups is necessary for the reduction of dehydroascorbic acid to ascorbic acid. The enzyme system of oxidation and decarboxylation of pyruvic acid is considered to be a sulfhydryl enzyme. At the same time, all enzyme poisons reacting with sulfhydryl groups substantially affect the activity of thiamine-containing enzymes (R. S. Vorob'yeva and S. V. Suvorov, 1961).

It should be kept in mind that the sensitivity of thiol enzymes to various chemical agents apparently varies, so that the effect of each of them is unique. Many are undoubtedly capable of reacting not only with thiol radicals but also with other chemical groups, and this gives a distinctive mark to the character of their effect (M. L. Belen'kiy and V. I. Rozengart, 1949). Para- and metachlorophenyl isocyanates cause a decrease in the amount of sulfhydryl groups in the blood serum. A decrease in the content of sulfhydryl groups in the liver was also observed (see Table 2). It is possible that chloroisocyanates interact with the reactive groups of proteins (sulfhydryl groups) and also with certain forms of thiamine.

Of late, attention has been focused increasingly on the study of carbohydrate-protein complexes and products of their splitting. Most of the techniques are based on the determination of the sialic acids (collective name for derivatives of neuraminic acid) entering into their composition, since sialic acids constitute the most reactogenic group of glycoproteins. Glycoproteins include hormones, enzymes, enzyme inhibitors and activators, and hormone carriers. Glycoproteins include many substances having a serological specificity (gamma globulins, complement components). The function of sialic acids in the body has thus far received little study, but the available data indicate that they participate in defensive mechanisms against the action of harmful agents (A. A. Titayev et al., 1964).

We used the determination of the content of sialic acids in the blood serum as an indicator of the nonspecific changes resulting from the effect of chemical substances on the rat organism. Chloroisocyanates caused a 70-50% statistically significant increase in the content of sialic acids relative to the control (see Table 2).

In substantiating the maximum permissible concentrations of atmospheric pollutants, the excretion of coproporphyrins with the urine is frequently studied as an indicator of general biological shifts in the body. A marked decrease of the content of coproporphyrins in the urine took place under the influence of chloroisocyanates. The confidence factor of the changes caused by parachlorophenyl isocyanate was 99%, and metachlorophenyl isocyanate, 95%.

All the changes caused by the inhalation of chloroisocyanates were associated with shifts in the values of the rheobases and chronaxies of the extensors and flexors of the right rear shin (the determination was made by using a standard technique with the aid of an ISE-01 electronic stimulator). Against the background of an increase in the rheobase of the extensors, their chronaxy was shortened; the rheobase of the flexors decreased slightly without causing any appreciable changes of the chronaxy. These shifts lead to a disturbance of the normal ratios of the rheobases and chronaxies of the extensors and flexors: a change of the rheobases in opposite directions causes an increase of their ratio in antagonist muscles; the chronaxies of the extensors and flexors converge, causing an inversion of their ratio, which falls below unity (Fig. 3). More pronounced and stable changes occur under the influence of parachlorophenyl isocyanate.

According to the data of many authors (Yu. M. Uflyand, D. N. Markov, A. N. Magnitskiy, and others), the action of various unfavorable factors leads to an attenuation of the influence of the central nervous system on the periphery, and this effects a change in the values of the rheobase and chronaxy and an impairment of the interrelationships between antagonist groups of muscles.

In the course of a three-week recovery period, disturbances in the various systems of the experimental animals disappeared, indicating a functional character of the shifts observed.

In summarizing, it may be stated that the action of large concentrations of the substances studied gives rise to changes that indicate both a nonspecific action of the two chloroisocyanates and, to a certain extent, the ability of parachlorophenyl isocyanates to cause a sensitization of the organism.

Symptoms of the allergic effect of parachlorophenyl isocyanate were observed only during the first half of the exposure. It is probable that in a concentration of 0.03 mg/m$^3$, while stimulating the manufacture of antibodies, this substance simultaneously stimulates mechanisms which depress antibody formation and the allergic reaction. I. Ya. Uchitel' and E. L. Khasman (1964) suggested that the slight production of antibodies upon immunization of animals with polysaccharide complexes that, like many allergens, are not complete antigens, takes place because the doses of endotoxin capable of causing the formation of antibodies are sufficiently large to give rise to a stress reaction that can depress the production of a specific protein. This type of endotoxin action is observed, for example, in rats, animals which are particularly sensitive to the action of a stressor (P. F. Zdrodovskiy, 1964). This is apparently why rats are resistant to the action of allergens.

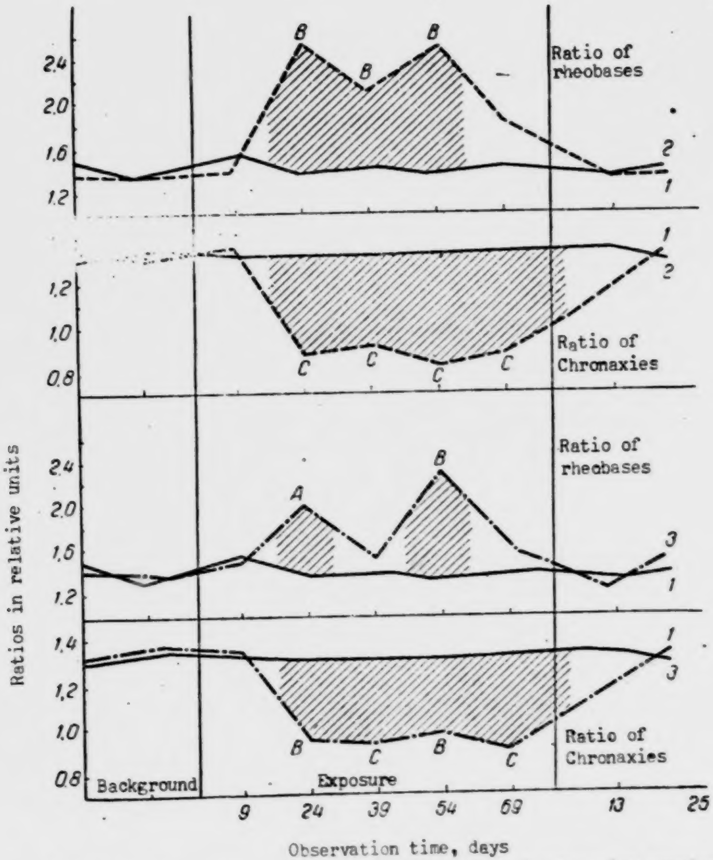

Fig. 3. Ratio of the rheobases and chronaxies of antagonist muscles during exposure to chloroisocyanates. Notation same as in Fig. 1.

Parachlorophenyl isocyanate has a somewhat similar effect. It should be remembered, however, that the intensity of the irritant is too low to classify this substance as a stressor in the generally accepted sense. The observed changes in the pattern of action of parachlorophenyl isocyanate in the course of the experiment should apparently be attributed to the duration and continuity of the effect. The fact that metachlorophenyl isocyanate has no such specific action may be ascribed to its lower toxicity.

## Conclusions

1. The low level of the active concentrations indicates a high toxicity of the chloroisocyanates. As shown by the results of the studies, parachlorophenyl isocyanate is the more toxic substance.

2. The toxic effect of both chloroisocyanates is chiefly manifested in a straining of the defensive reactions of the body. During the action of parachlorophenyl isocyanate (0.03 $mg/m^3$), there are certain signs of allergization of the animals, this being uncharacteristic of the meta isomer in the concentrations studied. In experimental studies of the effect of atmospheric pollutants, it is necessary to consider the possibility of a sensitizing action of chemical substances in low concentrations.

3. Differences in chemical structure are of unquestionable significance in the degree and character of the action of microconcentrations of chloroisocyanates.

4. The highest single and mean daily maximum permissible concentrations of para- and metachlorophenyl isocyanates are proposed at levels of 0.0015 and 0.005 $mg/m^3$, respectively.

## LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

# THE TOXICOLOGY OF LOW CONCENTRATIONS OF AROMATIC HYDROCARBONS

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 132-150, (1968).

The steady interest in benzene, toluene and xylene shown by toxicologists and hygienists is due to the fact that these substances thus far have not lost their industrial importance, possess a pronounced toxicity with a wide spectrum of effects and, despite a large number of studies, remain far from adequately studied. The toxicity of benzene, toluene and xylene has been studied (but not at all to the same extent) in acute and subacute experiments on animals and also in chronic experiments at the concentrations prevailing under industrial conditions and exceeding the existing maximum permissible levels for plant shop areas. The influence of low concentrations, i. e., concentrations equal to or below the existing maximum permissible ones for industrial buildings, has not been studied.

During the entire history of the toxicology of these substances, most attention was concentrated on the investigation of benzene. Considering it to be the most toxic of aromatic hydrocarbons of the benzene series, toxicologists have aimed most of their investigations at low benzene concentrations.

Yu. V. Novikov (1957) studied the effect of benzene in concentrations of 64 and 13 mg/m$^3$ during an exposure of 5½ months, at the rate of six hours six times a week. At the 64 mg/m$^3$ concentrations, the animals showed functional shifts in higher nervous activity; these shifts appeared during the 2nd-4th month of exposure and gradually progressed. The effect was manifested in the disinhibition of differentiating inhibition, extension of the latent period of conditioned reflexes to a bell and loss of motor conditioned reflexes to red light in rats of weak type, and also in the appearance of equalizing and paradoxical phases in other rats. Changes in the central nervous system were observed pathomorphologically. They were much less distinct in rats of the second group. No distinct changes were found in the morphological composition of the blood.

A. P. Volkova (1958) carried out a dynamic exposure of rabbits to benzene in concentrations of 50 and 20 mg/m$^3$ (three hours each in the course of three months). During the action of the first concentration, leucopenia, thrombopenia, anemia and reticulocytosis were observed in the animals; the phagocytic activity of the blood leucocytes decreased by 50%. At the 20 mg/m$^3$ concentration, a slight leucocytosis was observed in the blood of the animals; the phagocytic activity of the blood decreased by 40%. Numerous hemorrhages were observed anatomicopathologically in the internal organs, and considerable ones (as well as petechial hemorrages in the lungs) were found in the cortical and medullary substance of the kidneys.

A. I. Korbakova, S. N. Kremneva, N. K. Kulagina and I. P. Ulanova (1960) studied the effect of benzene concentrations of 20-40 mg/m$^3$ in the course of a 12-month chronic exposure (5½ hours six times a week). The animals used were cats, rabbits, and rats. In all the animals, the exposure caused undulatory and gradually progressive changes in the nervous system, and a tendency toward a depression of cholinesterase activity. Changes in the blood appeared after disorders in the nervous system and were manifested in a decrease of the total amount of thrombocytes, a certain shift to the left at the end of the exposure, and the appearance of young neutrophils.

The indicated studies are the only experimental ones on the effect of low benzene concentrations. No experiments have been conducted on the effect of low concentrations of toluene and xylene.

Another practically important and scientifically interesting problem is that of the dependence of the degree and character of the toxicity of a substance on the change of its chemical structure, i. e., the problem of the comparative toxicity of substances of a single series. Until now, the solution of this problem has been approached from the standpoint of the effect of these substances on the blood and blood-forming organs, this effect being considered the most dangerous and specific. Thus, not one official classification of chronic intoxication with benzene mentions changes in the nervous system. Most often, the problems of the interrelationship of specific and nonspecific reactions in a comparative evaluation of the effect of benzene and its homologs remained outside the scope of the researchers.

In recent years, papers dealing with the effect of the substances studied on the central nervous system have been published quite frequently. Thus, R. I. Yaroslavskaya (1952) noted that a decrease in the excitability of the cerebral cortex during the action of benzene takes place before the development of characteristic changes in the peripheral blood. G. E. Rozentsvit (1954) pointed out that the early stages of chronic benzene intoxication lead to disorders of the functional state of the cerebral cortex and of the cortical-subcortical neurodynamics. Disorders of the nervous system frequently precede changes in the blood and often are the only sign of intoxication. N. V. Revnova (1965) cited the data of an observation of 100 patients, in 19 of which the only manifestation of intoxication with benzene, toluene and xylene were disorders of the nervous system functional in character and defined as a neurasthenia syndrome with vegetative dysfunction.

The studies cited above (Yu. V. Novikov, 1957; A. I. Korbakova et al, 1960) also note an earlier and pronounced disturbance of the functions of the central nervous system during the action of benzene. These data indicate a major importance of disorders of the activity of the nervous system in the pathogenesis of occupational intoxications and the necessity of a comprehensive evaluation of all the changes arising under the influence of the substances studied.

Changes in the fine structures of the central nervous system and distortions of the biochemical processes give rise to disorders of the physiological functions. That is why in the prophylaxis of intoxications it is so important to study "nonspecific" reactions during exposure to low concentrations of toxic substances and to establish their thresholds.

The interest in the toxicology of low concentrations of chemical substances is explained by the possibility of not only their action on the human body under industrial conditions, but also their penetration of atmospheric air and action on the organisms of the population living in the vicinity of the industrial plants. The effect of the poison depends not so much on its concentration as on the duration of its action. Consequently, under the conditions prevailing in populated areas, the danger of contact with low concentrations increases considerably. The mode of action of polluted air of populated areas on the human body has been taken into account in the practical standardization of the mean daily maximum permissible concentrations of atmospheric pollutants, which specifies that a prolonged round-the-clock experiment must be carried out. The mean daily concentrations of toluene and xylene have not been established thus far, and the mean daily maximum permissible concentration of benzene requires an additional experimental verification.

The object of the present study was to obtain experimental data on the effect of benzene, toluene and xylene in concentrations below the existing maximum permissible ones for industrial buildings (the maximum permissible value for benzene is 20 mg/m$^3$ and for toluene and xylene, 50 mg/m$^3$), to evaluate them in a comparative manner, and also to obtain experimental material for substantiating the maximum permissible concentrations of these substances in the atmospheric air of populated areas.

To this end, we conducted a continuous 85-day exposure of 105 white male rats divided into 7 groups with 15 animals in each group. Pure air was supplied at a rate of 35 l/min into the chamber of the first group of animals (control), and air with an admixture of a certain amount of the substances studied was supplied to the remaining groups. The average concentrations in the chambers during the exposure and their fluctuations are shown in Table 1. The concentrations of the substances in the chambers were controlled daily. The average concentrations of the substances during the exposure were close to the calculated ones, with small indices of error of the arithmetic means. The air samples were taken and analyzed by using the techniques of M. V. Alekseyeva (1964).

Table 1

Concentrations of Benzene, Toluene and Xylene in the Chambers During Round-the-Clock Exposure

| Group | Substance | Concentration, mg/m$^3$ | | |
|---|---|---|---|---|
| | | Calculated | Average for the Period of Exposure | Range |
| First . . . | Control | — | — | — |
| Second . . . . | Benzene | 1,5 | 1,49±0,024 | 1,05—1,90 |
| Third . . . | | 15,0 | 15,07±0,233 | 12,0—18,5 |
| Fourth . . | Toluene | 0,6 | 0,59±0,009 | 0,43—0,78 |
| Fifth . . . . | | 15,0 | 14,65±0,162 | 12,0—16,6 |
| Sixth . . . | Xylene | 0,2 | 0,23±0.006 | 0,16—0,38 |
| Seventh . . . | | 15,0 | 14,60±0,168 | 12,1—17,4 |

The concentrations of benzene (1.5 mg/m$^3$), toluene (0.6 mg/m$^3$), and xylene (0.2 mg/m$^3$) were taken at the level of subthreshold concentrations for the reflex effect (I. S. Gusev, 1965). These concentrations are found most frequently in atmospheric air, and the study of the possibility of their resorptive effect is of definite interest from the standpoint of a sanitary evaluation of the pollution of air in populated areas.

The concentration of the substances studied, 15 mg/m$^3$ (10 times the subthreshold value for the reflex effect of benzene), was taken at the same level for the purpose of the clearest possible comparative evaluation of their toxic effect.

Before the exposure, the animals were kept in chambers for two weeks to get them to adapt to the conditions of the experiment and to record the background data on the indices of interest to us. The exposure was followed by a one-month recovery period.

The animals were fed twice a day in accordance with the standards set forth by the instructions of the Ministry of Health of the USSR, with the daily introduction of cereals, meat, milk, vitamins, vegetables, bread, and feed pellets. The animals received water in unlimited amounts.

In order to reinforce the changes detected and to identify latent signs of intoxications, during the second half of exposure, a 15-day partial starving of the animals was conducted, which is considered in toxicology as one of the methods of functional loading that disturbs the existing systems of complex reflex regulations. Cereals, meat, milk and bread were excluded from the ration. The animals were fed once: feed pellets and vegetables.

In the course of the experiment, observations were made on the dynamics of the weight and the general condition of the experimental animals. Considering that the substances studied were poisons for the central nervous system and blood, we concentrated our attention on these functions. Analyses of the ratios of motor chronaxies of antagonist muscles and of the cholinesterase activity of whole blood served as indices of the effect on the central system. The effect on the blood was evaluated from changes in the total number of leucocytes and differential white count of the rats.

The results of all the observations were treated statistically by the range method, with the determination of the degree of significance of the changes obtained (A. M. Merkov, 1960).

We began the studies on healthy adult rats with an average weight of 220 g (the weight range from 180 to 260 g in the groups). During the exposure, the external appearance of the experimental animals did not differ appreciably from that of the controls. Animals of the third, fifth and seventh groups, exposed to the substances studied in a concentration of 15 mg/m$^3$, were characterized by an increased excitability, restlessness, unwillingness to be handled, and agressiveness, manifested particularly in rats of the seventh group (xylene).

Animals in all the groups willingly accepted food and gained weight normally. They were weighed once every 20 days. The changes were evaluated from the percent increase over the initial weight. In the first half of the exposure, the weight of rats of all the groups did not differ from that of the controls. During the period of experimental starving, animals of all the groups lost weight; the most pronounced weight loss was observed in rats of the seventh group. Subsequently, their weight lagged slightly behind the weight of the control animals, but the data of the change were not statistically significant.

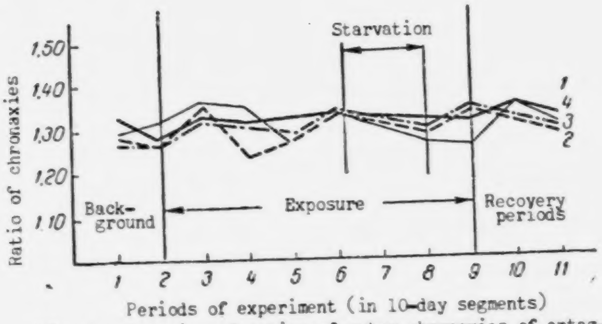

Fig. 1. Dynamics of ratios of motor chronaxies of antagonist muscles in animals of the second, fourth, sixth and control groups.

1 - control group; 2 - second group; 3 - fourth group; 4 - sixth group

Muscular chronaxy is subject to cortical regulatory influences. Changes of the motor chronaxy are due to disturbances of the functional state of the cerebral cortex and, in particular, of the motor analyzer of the cortex* (U. Sh. Akhmerov, 1956).

The level of the ratios of motor chronaxies of antagonist muscles is completely determined by central influences, and its change is regarded as an attenuation of the subordinating influences of the cerebral cortex.

Our studies of the motor chronaxy and rheobase were carried out with an ISE-01 pulsed electronic stimulator on five rats of each group, once every ten days. These indices were determined on the flexors and extensors of the right rear shin. The response was recorded from the twitch of the paw. Average results of the ratios of chronaxies of antagonist muscles for the groups of experimental animals were treated statistically with reference to the analogous indices of the control (Table 2).

As is evident from this table, the average ratios of motor chronaxies of antagonist muscles in animals of the second, fourth and sixth groups during the action of subthreshold concentrations of benzene, toluene and xylene in terms of the reflex effect did not differ appreciably from the analogous indices of the control group. Statistical treatment of the material did not show any significant changes in any of the groups (Fig. 1).

The action of the substances studied in concentrations of 15 mg/m$^3$ caused distinct and profound changes in the ratios of chronaxies. As early as the 20th day of exposure, an inverted ratio was observed in animals of the seventh group, whereas a pronounced decrease of the ratios of chronaxies was noted in animals of the fifth group. In rats of the third group (benzene), statistically significant changes of these indices were noted ten days later. Subsequently, these changes became accentuated, retaining high confidence factors until the end of the exposure. During the partial experimental starving, the degree of inversion of the ratios of chronaxies of antagonist muscles in the groups of animals under consideration increased somewhat. After the starvation, a certain increase of the ratios was observed in animals of the third group, whereas a further accentuation of the changes took place in animals of the fifth and seventh groups, particularly marked in animals of the latter (Fig. 2). An interesting sequence was established during the recovery period. A rapid recovery was observed in animals of the third group; as early as the 20th day of this period, the changes became insignificant, and toward the end of the month the ratio of chronaxies returned to its original level. The indices under study returned to normal somewhat more slowly in rats of the fifth group; the significance of the changes disappeared toward the end of the month, but the level of the ratios still lagged behind the control. A very slow recovery was noted in the seventh group of animals; an inverted ratio of chronaxies with a high degree of significance of the detected changes was preserved until the end of the recovery period.

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* Editor's note: motor cortex.

Table 2

Change in the Ratios of Motor Chronaxies of Antagonist Muscles in the Course of Chronic Exposure of Animals to Low Concentrations of Benzene, Toluene, and Xylene.

| Group | Average Values for Groups of Ratios of Chronaxies of Antagonist Muscles | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Background | | Exposure | | | | | | Recovery | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| First . . | 1.33 | 1,27 | 1.33 | 1,31 | 1,32 | 1,33 | 1,31 | 1,32 | 1,35 | 1,32 | 1,33 |
| Second . . | 1,28(c) | 1,26(o) | 1.34(o) | 1,23 (o) | 1,27 (o) | 1,34 (o) | 1,28 (o) | 1,33 (o) | 1,31 (o) | 1,28 (o) | — |
| Third . . | 1,33(o) | 1.36(o) | 1.32(o) | 1,24 (o) | 0,88 (b) | 0,76 (c) | 0,70 (c) | 0,73 (c) | 0,93 (b) | 1,18 (o) | 1,30 (o) |
| Fourth . | 1.27(c) | 1.2[illegible](o) | 1.32(o) | 1,30 (o) | 1,28 (o) | 1,33 (c) | 1,30 (o) | 1,34 (c) | 1,33 (o) | 1,29 (o) | — |
| Fifth . . . | 1,29(o) | 1.36(o) | 1.29(o) | 1,08 (b) | 0,82 (c) | 0,74 (c) | 0,69 (c) | 0,63 (c) | 0,85 (b) | 1,09 (a) | 1,20 (o) |
| Sixth . . | 1,29(o) | 1.32(o) | 1.36(o) | 1,35 (o) | 1,27 (o) | 1,32 (o) | 1,27 (o) | 1,26 (o) | 1,35 (o) | 1,30 (o) | — |
| Seventh . . | 1,27(o) | 1.31(o) | 1,31(o) | 0,98 (b) | 0,73 (c) | 0,79 (c) | 0,69 (c) | 0,57 (c) | 0,63 (c) | 0,87 (b) | 0,97 (b) |

Note. In this and the following tables, the letters a, b, and c denote the confidence factors of the data obtained, corresponding to the probability of an error of not more than 5, 1, and 0.1% respectively. o - not significant.

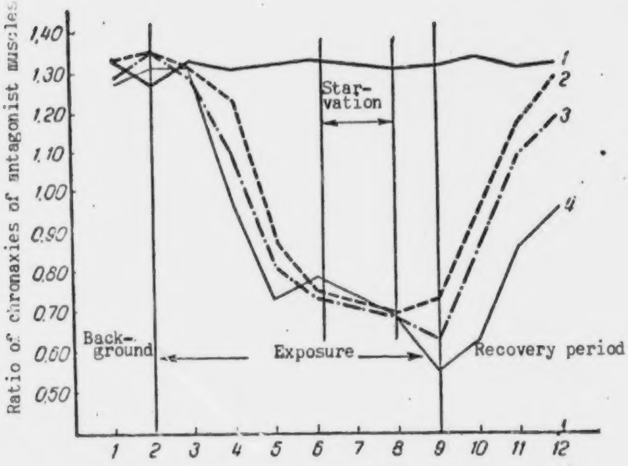

Fig. 2. Dynamics of ratios of motor chronaxies of antagonist muscles in animals of the third, fifth, seventh and control groups.
1 - control group; 2 - third group; 3 - fifth group; 4 - seventh group.

The dynamics of ratios of motor chronaxies of antagonist muscles during the action of benzene, toluene and xylene in concentrations of 15 mg/m$^3$ indicates the extent and manifestation of the changes taking place in the central nervous system.

Cholinesterase is one of the antiregulatory factors participating in the pathogenesis of all the vegetative disorders associated with an increased formation of acetylcholine; the ability of the organism to adapt is explained by the humoral mechanism of self-regulation (D. Ye Al'pern, 1958).

Studies of the cholinesterase activity of whole blood were made by using the Fleischer-Pope photometric method (1954) modified by the department of communal hygiene of the First Moscow Medical Institute im. M. I. Sechenov. The modifications introduced into the Fleischer-Pope method concerned the sequence of the control setup and the number of control samples.

Having observed that blood per se does not give a color in solutions, the staff of the department proposed that duplication of controls with blood for each of the experimental samples be excluded, and replaced with a single control for a given experiment. The sequence of the subsequent procedure remained unchanged.

The observations were made on five rats of each group, twice a month over the course of the entire study. The blood was taken from the tail vein of the animals. Average values of the cholinesterase activity of whole blood according to groups of animals and the results of the statistical treatment are presented in Table 3.

As is evident from this table, the cholinesterase activity showed marked fluctuations in all the groups in the course of the exposure.

For greater clarity, the results of the study of cholinesterase activity are given in percent of the control (Fig. 3).

No significant changes were detected in the statistical treatment of data of the second, fourth and sixth groups. The cholinesterase activity of rats of the third, fifth and seventh groups during the first half of the exposure showed a depressant tendency that was statistically significant for the seventh group only. During the second half of the exposure, particularly the starvation period, an increase in cholinesterase activity significant only for rats of the fifth group was observed.

Thus, the study of the cholinesterase activity of whole blood in the course of round-the-clock exposure of the animals to benzene, toluene and xylene did not yield any clear-cut data that would enable one to use the effect of the substances studied on the central nervous system in a comparative evaluation based on this index.

The lack of precise and definite results in no way indicates an absence of the effect of benzene, toluene and xylene in a concentration of 15 $mg/m^3$ on the state of the central nervous system. This method, based on the determination of total cholinesterases (specific and pseudo) cannot compare with chronaximetry in sensitivity. In our view, a given intensity of the action of the substances studied may affect the mediator factors of nervous stimulation in animals of heightened sensitivity (even for a given duration of contact), as illustrated by a marked and stable depression of cholinesterase activity in rats Nos. 9 and 10 (seventh group). Benzene, toluene and xylene are not specific inhibitors of cholinesterase and it is entirely possible that a longer contact time is necessary for the manifestation of a more pronounced action of these substances on the mediator functions.

Recent studies by A. P. Volkova (1958), A. I. Korbakova et al. (1960), Kuhbock and Lachnit (1962), Deichmann and Villiam (1963) and others indicate more frequent and earlier damage of the white blood cells during the action of low benzene concentrations.

We determined the total number of leucocytes twice a month on five rats of each group. The blood was taken from the tail vein by incision, asepsis rules being observed. The blood was withdrawn by using N. M. Nikolayev's sampling method. The counting was done in a Goryayev chamber.

Table 3

Change in the Cholinesterase Activity of Whole Blood of Animals in the Course of Chronic Exposure to Low Concentrations of Benzene, Toluene and Xylene.

| Group | Average Indices for Groups According to Periods of Experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Background | | Exposure | | | | | Recovery |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| First | 204,6 | 174,2 | 170,8 | 225,0 | 200,0 | 162,8 | 242,8 | 233,3 |
| Second | 217,4 (o) | 186,8 (o) | 170,2 (o) | 231,4 (o) | 208,6 (o) | 175,8 (o) | 250,8 (o) | 210.0 (o) |
| Third | 204.2 (o) | 188,0 (o) | 172,8 (o) | 204,0 (o) | 200,2 (o) | 174,6 (o) | 233.2 (o) | 228,3 (o) |
| Fourth | 192,8 (o) | 162,8 (o) | 160,0 (o) | 194,4 (o) | 196,0 (o) | 173,0 (o) | 214,6 (o) | 200,0 (o) |
| Fifth | 197,0 (o) | 184,8 (o) | 173.2 (o) | 199,0 (o) | 185,8 (o) | 223,6 (a) | 250.8 (o) | 228,3 (o) |
| Sixth | 231,4 (o) | 180,0 (o) | 195,4 (o) | 198,6 (o) | 227,4 (o) | 207,0 (o) | 237,6 (o) | 215.0 (o) |
| Seventh | 221,6 (o) | 163,6 (o) | 176,0 (o) | 166,0 (A) | 185,4 (o) | 184,0 (o) | 215.2 (o) | 183,3 (o) |

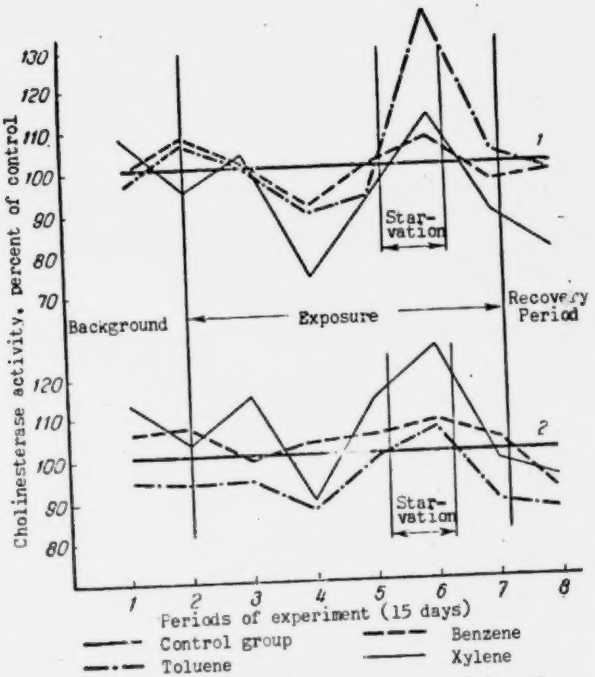

Fig. 3. Change in the cholinesterase activity of whole blood of animals in the course of round-the-clock exposure.

1 - cholinesterase activity of animals of the third, fifth, seventh, and control groups; 2 - cholinesterase activity of animals of the second, fourth, sixth, and control groups.

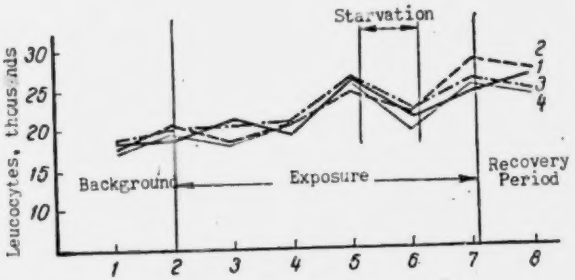

Fig. 4. Total number of leucocytes in the peripheral blood of animals of the second, fourth, sixth and control groups. Notation same as in Fig. 1.

Table 4

Change in the Total Number of Leucocytes in the Peripheral Blood of Animals During the Action of Low Concentrations of Benzene, Toluene and Xylene.

| Group | Average Indices for Groups According to Periods of Experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Background | | Exposure | | | | | Recovery |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| First | 18 280 | 18 800 | 20 550 (o) | 19 670 | 26 530 | 21 230 | 24 780 | 26 630 |
| Second | 17 750 (o) | 20 620 (o) | 18 220 (o) | 20 050 (o) | 24 860 (o) | 21 370 (o) | 28 500 (o) | 27 000 (o) |
| Third | 18 210 (o) | 19 460 (o) | 17 930 (o) | 18 210 (o) | 16 690 (b) | 15 460 (o) | 20 140 (o) | 24 266 (o) |
| Fourth | 18 420 (o) | 20 260 (o) | 20 210 (o) | 20 780 (o) | 26 670 (o) | 22 420 (o) | 25 890 (o) | 24 416 (o) |
| Fifth | 19 610 (o) | 20 550 (o) | 19 990 (o) | 19 490 (o) | 25 000 (o) | 17 050 (o) | 25 270 (o) | 25 400 (o) |
| Sixth | 17 050 (o) | 19 350 (o) | 18 130 (o) | 20 100 (o) | 25 090 (o) | 19 630 (o) | 25 110 (o) | 24 356 (o) |
| Seventh | 20 150 (o) | 20 870 (o) | 19 690 (o) | 27 890 (a) | 27 060 (o) | 27 830 (a) | 36 030 (o) | 30 866 (o) |

Average indices of the total number of leucocytes according to groups of animals for the period of chronic exposure and the results of the statistical treatment are listed in Table 4.

The total number of leucocytes in animals of the second, fourth, and sixth groups which inhaled subthreshold concentrations in terms of the reflex effect of the substances studied did not substantially differ from the control in the course of exposure. No significant changes were detected by the statistical treatment (Fig. 4).

In animals of the third, fifth, and seventh groups (at a concentration of the substances studied of 15 mg/m$^3$), these indices displayed some marked changes. Thus, the total number of leucocytes in the peripheral blood of animals of the third group had decreased by the 45th day; the significance of the changes obtained was of degree B. During the period of experimental starvation, these changes were somewhat accentuated, but their significance was not confirmed statistically. Subsequently, adaptation of the white blood cells to benzene was observed, with complete normalization by the 20th day of the recovery period. In the fifth group of rats, no distinct statistically significant changes were established in the total number of leucocytes during the course of the experiment. During the starvation period there was noticed a tendency toward a drop in their level. More persistent changes on the data of the indices was observed in the animals of the seventh group. As early as the end of the first month of exposure, a statistically significant increase in the number of leucocytes was observed (degree A). Subsequently, the number of leucocytes in the blood of animals of this group remained higher during the starvation period as well, and the significance of the changes was confirmed statistically. A marked increase in the number of leucocytes was noted during the last period of exposure, but because of the large range of the indices in the group itself, which we attributed to different individual sensitivities of the rats to xylene, these changes were not statistically significant. By the 20th day of the recovery period, the total number of leucocytes in the blood of rats of the seventh group had decreased markedly, but remained above these indices for the control group (Fig. 5).

The collection of blood for studying its picture was made simultaneously with that of blood for counting the total number of leucocytes, and on the same animals. The analysis involved the use of the technique of N. G. Alekseyev, whereby the blood smear was stained first in a specially prepared solution of Azur II, and the smears thus prepared were counterstained with the Romanovskiy-Giemsa staining solution. In order to derive the picture, we counted units of 200 cells over the entire surface of the specimen (continuous count method).

Analysis of the material obtained and statistical treatment of the data according to the separate formed elements (absolute values) make it possible to establish the following:

a) In the peripheral blood of animals of the second, fourth, and sixth groups, no clear-cut changes of the formed elements were noted;

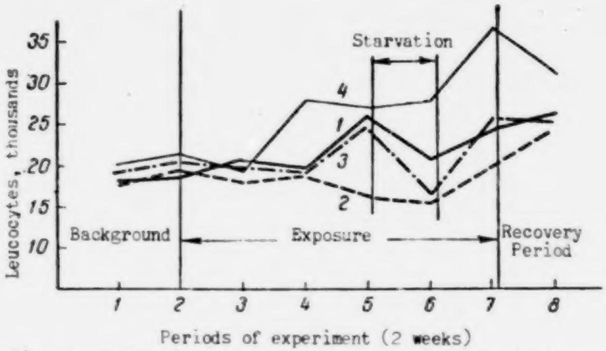

Fig. 5. Change in the total number of leucocytes in the peripheral blood of animals of the third, fifth, seventh, and control groups. Notation same as in Fig. 2.

b) Unstable leucopenia during the action of benzene in a concentration of 15 mg/m$^3$ was accompanied by neutropenia with the appearance of staff cells, a certain decrease in the number of eosinophils and monocytes, with a relatively constant number of leucocytes.

c) An extended action of toluene in a concentration of 15 mg/m$^3$ causes neutropenia, the appearance of isolated staff cells, and relative lymphocytosis in the blood of experimental rats.

d) Leucocytosis during the action of xylene in a concentration of 15 mg/m$^3$ is associated with neutropenia, absolute lymphocytosis, an increase in the number of monocytes, and the appearance of staff cells.

The above-indicated changes were not pronounced. The shift to the left did not go beyond the appearance of isolated staff cells in the blood. We regard the changes obtained as initial functional shifts. At this stage, these changes were essentially compensated by the mobilization of defensive reactions.

The results of pathomorphological studies constitute a valuable confirmation of the effect of the substance studied, and aid in the determination of its extent, nature and direction.

In order to carry out the pathomorphological studies, three rats of each group were killed by decapitation on the last day of exposure. Their lungs, heart, liver, spleen, kidneys, and brain were examined. Statistical treatment of data on the relationship of the weight of the internal organs to the total weight of the animals did not yield any significant changes.

The results of pathomorphological studies* lead to the conclusion that the concentrations of 1.5 mg/m$^3$ benzene, 0.6 mg/m$^3$ toluene and 0.2 mg/m$^3$ xylene do not cause any perceptible morphological changes in the organs or tissues of the experimental animals; benzene, toluene and xylene in a concentration of 15 mg/m$^3$ cause distinct changes of essentially the same type, at the level functional shifts, consisting in circulatory hemodynamic disorders in the internal organs (lungs, liver, heart, kidneys, spleen, brain), a decrease in the glycogenic activity of the liver, its lipodystrophy, an increased breakdown of erythrocytes in the spleen with deposition of hemosiderin, and partial tigrolysis in the neurons of the brain.

Thus, the above-mentioned functional changes in animals exposed to benzene, toluene and xylene in a concentration of 15 mg/m$^3$ have received an ample pathomorphological corroboration.

A unified approach to the evaluation of toxicity using the same highly sensitive indices, single stages of investigation and methods of quantitative evaluation of the effects observed enabled us to obtain data characterizing the comparative toxicity of the substances studied.

## Conclusions

1. A continuous 85-day exposure of white rats to benzene, toluene and xylene in a concentration of 15 mg/m$^3$ causes marked changes in the central nervous system and blood, reflected pathomorphologically in the internal organs of the experimental animals.

2. Most noticeable are changes in the central nervous system, which occur before the changes in the blood and are more pronounced and more stable. The degree of manifestation and stability of the changes in the central nervous system increases with the number of methyl groups in the benzene ring.

3. We regard the changes obtained in the total number of leucocytes and in the white count of the experimental animals (less stable for benzene and toluene) as initial functional shifts. At the level in question, it is impossible to carry out a precise comparative evaluation of these changes.

4. There is no reason to regard benzene as more toxic than toluene or xylene.

5. The concentrations of 0.6 mg/m$^3$ toluene and 0.2 mg/m$^3$ xylene, which preclude the possibility of not only reflex reactions but also of resorptive

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* The pathomorphological studies were carried out by the scientific collaborator O. V. Kolbasova under the direction of Doctor of Medical Sciences V. P. Osintseva, director of the laboratory of pathomorphological studies of the A. N. Sysin Institute of General and Communal Hygiene of the Academy of Medical Sciences of the USSR, both of whom the author sincerely thanks.

action under conditions of a prolonged constant contact, may be recommended as the mean daily maximum permissible values for atmospheric air.

6. For the time being, until the problem of the possibility of accumulation during the action of low concentrations of benzene is solved, we consider it advisable to leave its mean daily maximum permissible concentration at the earlier level, 0.8 $mg/m^3$, which amounts to one-half the inactive value which we have established.

## LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

# CHRONIC ACTION OF LOW CONCENTRATIONS OF ACROLEIN IN AIR ON THE ORGANISM

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 122-135, (1967).

In industry, acrolein* is prepared by oxidizing propylene over CuO or by vapor phase condensation of acetaldehyde with formaldehyde in the presence of $Zn_3(PO_4)_2$. Considerable quantities of acrolein are used in the production of glutaraldehyde and methionine.

Acrolein is a major product of glycerin synthesis. It escapes into the atmosphere from plants where glycerin is subjected to a high temperature (130-180°C.): during the manufacture of oilcloth and linoleum, in the core sections of foundry shops, in the production of insulation in the electrical industry. In recent years the presence of acrolein in the exhaust gases of motor transport has been demonstrated.

In atmospheric air and in the air of apartments around the Leningrad Fat Plant, V. Z. Yas'kova found very high concentrations of acrolein which in all probability were due to the use of a nonspecific method of determination of acrolein.

M. M. Plotnikova (1960) determined acrolein in atmospheric air around a chemical and an oil-chemical plant. At a distance of 100 m from the oil-chemical plant, the highest concentrations were 2 $mg/m^3$, in all the samples the amount of acrolein was above the maximum permissible concentration (0.3 $mg/m^3$), and at a distance of 1000 m, the maximum concentrations were 0.64 $mg/m^3$. Around the chemical plant (with the oil-boiling shop as the source of pollution), at a distance of 200 m, the highest concentration was 20 $mg/m^3$, and at 1500 m, 0.3 $mg/m^3$.

The effect of acrolein on the human organism is manifested by a marked irritation of the mucous membranes and a certain general toxic effect (N. V. Lazarev, 1954). The maximum permissible concentration of acrolein for shops is 0.002 mg/l.

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* See article by M. M. Plotnikoya in the Collection "Maximum Permissible Concentrations of Atmospheric Pollution", Prof. V. A. Ryazanov, Ed., 1960, vol. 4.

To validate the highest single maximum permissible concentration of acrolein in atmospheric air, M. M. Plotnikova determined the odor threshold. The minimum perceptible concentration was found to be 0.8 mg/m$^3$. The threshold of the reflex effect, determined by the method of optical chronaxy, was 1.75 mg/m$^3$, and that of the light sensitivity of the eye, 0.6 mg/m$^3$. The highest single maximum permissible concentration of acrolein in atmospheric air was established at a level of 0.3 mg/m$^3$. The mean daily maximum permissible concentration of acrolein (0.1 mg/m$^3$), adopted on the basis of a calculation, has not been experimentally validated thus far.

On the recommendation of the Committee on Sanitary Protection of Atmospheric Air, we studied the effect of low acrolein concentrations on the organism of experimental animals. A round-the-clock chronic exposure of white rats in three chambers (with a capacity of 100 l each) was carried out for this purpose. Group IV was the control. The round-the-clock exposure was carried out over the course of two months. There were 10 rats in each chamber.

Acrolein in the air of the exposure chambers was determined by the tryptophane method by D. P. Senderikhina, modified by M. M. Plotnikova. The method is based on the fact that on reacting with acrolein, tryptophane forms a stable violet color. The sensitivity of the method is 0.002 mg in 2 ml of solution. The specificity of the method is of no major significance for our studies (M. V. Alekseyeva, 1962).

In the course of exposure in the first chamber (group I), the average acrolein concentration was found to be 1.52 ± 0.05 mg/m$^3$ with fluctuations from 2 to 1 mg/m$^3$, in the second chamber (group II) 0.51 ± 0.02 mg/m$^3$ with fluctuations from 0.22 to 0.77 mg/m$^3$, and in the third chamber (group III) 0.15 ± 0.01 mg/m$^3$ with fluctuations from 0.1 to 0.3 mg/m$^3$. Group IV was the control.

In order to validate the mean daily maximum permissible concentration of acrolein in atmospheric air, the following factors were determined in the experimental animals during the exposure: dynamics of weight and behavior; conditioned reflex activity; changes in the activity of whole blood cholinesterase; excretion of coproporphyrin with urine; percent content of the number of fluorescent* leucocytes and histopathological changes in the organs and tissues of animals which died from the effects of acrolein during exposure, and in some of the rats sacrificed after the end of exposure.

At the end of the first week of exposure, the animals of group I (1.52 mg/m$^3$) had a sickly appearance, became sluggish and apathetic, and their fur turned dull. The animals ate poorly. Their condition gradually worsened, and for this reason the exposure in this chamber was discontinued on the 24th day. During this period, all 5 rats in which the higher nervous activity was studied died, and of the remaining animals in the experiment, only two rats perished. No visible changes were observed in the behavior and condition of rats of groups II, III and IV.

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* Editor's note: For the Russian use of the terms "luminescent" and "luminescence" in this paper, we have substituted "fluorescent" and "fluorescence", on the basis of the definitions of these terms.

All the rats were weighed once a week during the exposure. The results are shown in Table 1.

Table 1

| Group | Acrolein Concentration $mg/m^3$ | Exposure (end) | Recovery Period |
|---|---|---|---|
| I | 1,52 | 90,7 (a)[1] | 108,7 (c) |
| II | 0,51 | 114,7 (b) | 121,7 |
| III | 0,15 | 128,3 | 128,9 |
| IV | Control | 129,1 | 134,8 |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%

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[1] Comparison with the control is given on the basis of the condition on 24 April, date on which the exposure was discontinued.

In animals of group I (1.52 $mg/m^3$), a rapid loss of weight was observed; when it was necessary to discontinue the exposure, the weight had decreased by 14% as compared with the initial weight. The weight decrease in animals of this group also continued after the exposure was discontinued. At the end of the first week of the recovery period, the weight loss was 25%. The average weight of the rats reached its original value only at the end of the 5th week of the recovery period, then began to increase gradually.

Statistically significant weight changes in the direction of a decrease were also observed in rats of group II (0.51 $mg/m^3$).

There were no statistically significant weight changes in the experimental animals of group III (0.15 $mg/m^3$).

To establish the maximum permissible concentrations of chemical substances in atmospheric air, a number of authors used the conditioned reflex method (Yu. V. Novikov, N. F. Izmerov, M. I. Gusev and K. N. Chelikanov, Ya. G. Dvoksin, V. N. Kursanov, and others). The studies showed that changes in the functions of the cortex of the cerebral hemispheres take place very rapidly. Early signs of disturbance of the higher nervous activity are phase states, facilitation of differentiation, loss of certain reflexes and finally, disconnection of all reflexes of the stereotype. In grave injuries, the natural conditioned reflex to the appearance and odor of food disappears. The recovery period may last a month and sometimes longer.

Essentially, the change in the higher nervous activity during the action of toxic substances is manifested in a steady weakening of the stimulating and inhibiting processes followed by the development of protective inhibition. The sensitivity of the method of conditioned reflexes is very high. The maximum permissible concentrations of benzene, mercury, and lead for plant shops earlier established in a study of their action on experimental animals during a chronic exposure with the aid of the method of conditioned reflexes were found to be many times greater than the threshold of their action.

However, the procedure used earlier for studying conditioned reflexes in Kotlyarevskiy's chamber is extremely laborious and time-consuming. We used an accelerated procedure for studying the higher nervous activity in white rats, modified by Ya. G. Dvoskin (1961). A total of 18 white male rats weighing from 64 to 102 g were used in the experiment; 5 each from groups I, II, and III, and 3 from the control group. In all the animals, the stereotype was developed in the following sequence: bell, light; light, bell.

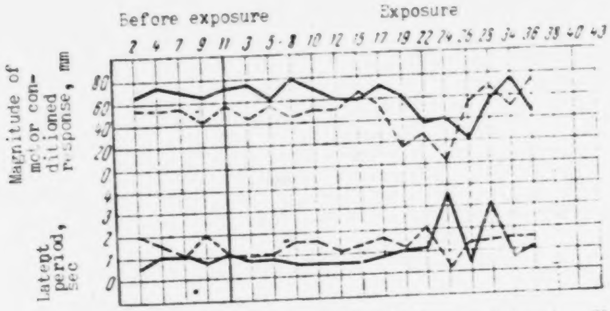

Fig. 1. Effect of acrolein on the conditioned reflex activity of rat No. 12 (concentration 1.52 mg/m$^3$).

The most profound changes in higher nervous activity were observed in rats of group I (1.52 mg/m$^3$). After the start of exposure, the magnitude of the motor conditioned response compared with the control decreased to both the bell and light. This was followed by the appearance of phase states. The conditioned reflex activity of one of the rats of this group is shown in Fig. 1.

Changes in the conditioned reflex activity were also observed in rats of group II (0.51 mg/m$^3$), but in a much less distinct form (Fig. 2).

In rats of group III (0.15 mg/m$^3$) and in the control group, no changes were detected in the conditioned reflex activity, as is evident from Figs 3 and 4. These figures show that in animals of groups III and IV, the normal strength relationships to the weak and strong stimuli and also the latent period were preserved during the entire study.

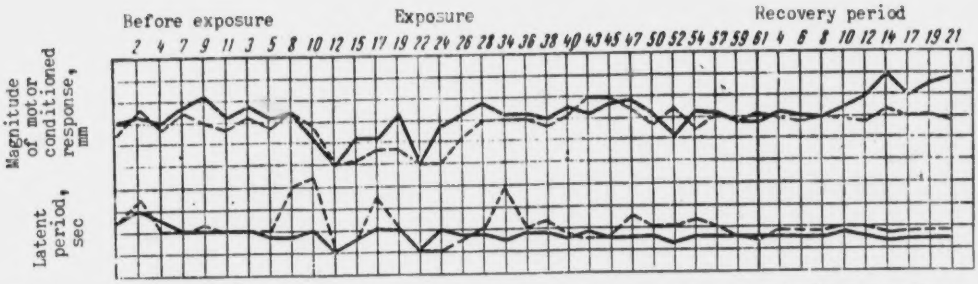

Fig. 2. Effect of acrolein on the conditioned reflex activity of rat No. 19 (concentration 0.51 mg/m³).

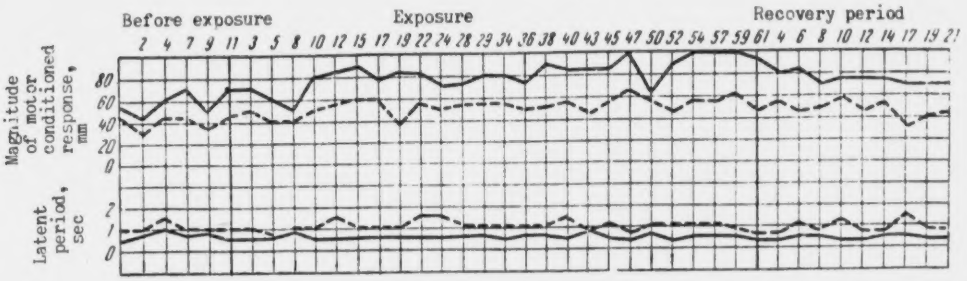

Fig. 3. Effect of acrolein on the conditioned reflex activity of rat No. 8 (concentration 0.15 mg/m³).

As was indicated above, rats of group I died from the effects of acrolein, and it was therefore impossible to follow the period of higher nervous activity in them. In rats of group II, the restoration of the activity of the cerebral cortex took place on the 10th day after the end of exposure.

The change in the activity of whole blood cholinesterase is directly related to a disturbance of the functional state of the nervous system (Ye. B. Babskiy and A. A. Kirillov, 1938; D. Ye. Al'pern, 1958).

In addition, an increase in cholinesterase activity is an expression of shifts in the functional state of the organism as a whole.

In the last few years, the method of determination of cholinesterase activity has found wide applications in the study of the effect of a number of substances in connection with the establishment of their maximum permissible concentrations. In studies directed by Prof. V. A. Ryazanov (G. I. Solomin, 1961; D. G. Odoshashvili, 1962; Li Sheng, 1961; R. U. Ubaydullayev, 1962; P. G. Tkachev, 1963; V. A. Chizhikov, 1963; V. I. Filatov, 1963, and others) it was shown that a change in cholinesterase activity is caused by concentrations 10-20 times smaller than the maximum permissible ones for industrial plants.

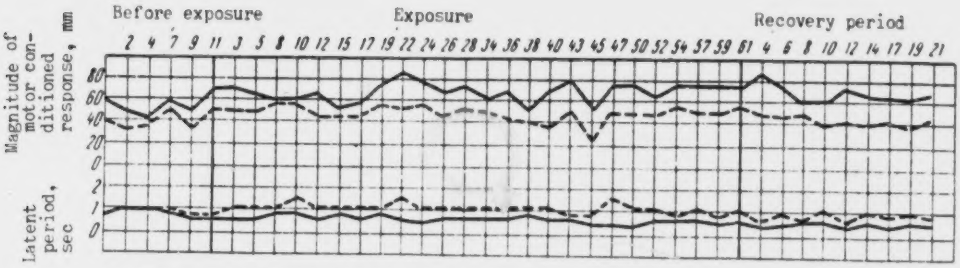

Fig. 4. Conditioned reflex activity of rat No. 5 (control group).

In our work, the activity of blood cholinesterase in white rats was determined by Pokrovskiy's method (1953) modified by A. P. Martynova (1957).

Essentially, this method consists in the hydrolysis of acetylcholine, determined by the time of change in the color of the indicator as a result of the pH shift. The cholinesterase activity was determined once a week in 20 rats (5 each from 4 groups). The results of the study are shown in Table 2.

The time of acetylcholine hydrolysis in the control group was 38.7-40.3 minutes. In rats of group I (1.52 mg/m$^3$), on the 15th day since the start of exposure, a significant decrease in blood cholinesterase activity was observed: The time of acetylcholine hydrolysis increased to 61 minutes. On the 24th day, the exposure of the animals in this chamber was discontinued, then the first study was made after 2 days, and the cholinesterase activity was found to remain low. A return of the cholinesterase activity to normal was recorded on the 2nd day after the exposure was discontinued.

A decrease in cholinesterase activity was also observed in rats of group II (0.51 mg/m$^3$). Statistically significant changes were recorded the first time on the 34th day of exposure and reached a maximum on the 41st day of the experiment. The time of acetylcholine hydrolysis began to decrease gradually even before the exposure was discontinued, and reached normal indices on the 10th day of the recovery period.

In animals of group III (0.15 mg/m$^3$) and the control group, no changes in the activity of whole blood cholinesterase were observed during the entire experiment.

Table 2

Time of Acetylcholine Hydrolysis in White Rats (minutes)

| Group | Concentration of Acrolein, mg/m$^3$ | Before Exposure | | | | | Exposure | | | | Recovery Period | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 18/III | 22/III | 8/IV | 15/IV | 24/IV | 6/V | 13/V | 20/V | 28/V | 11/VI | 19/VI |
| I | 1,52 | 37,4 | 38,4 | 39,3 | 61,0 (c) | 56,0[1] (b) | — | 44,0 | — | 40,0 | 40,5 | 39,7 |
| II | 0,51 | 41,4 | 40,6 | 40,8 | 41,5 | 40,8 | 47,4 (a) | 52,0 (c) | 48,4 (b) | 44,7 (a) | 38,5 | 39,5 |
| III | 0,15 | 40,4 | 39,6 | 40,4 | 40,5 | 40,0 | 40,0 | 41,2 | 39,3 | 39,9 | 40,0 | 39,5 |
| IV | Control | 39,1 | 39,0 | 38,7 | 40,3 | 39,2 | 39,2 | 39,4 | 39,8 | 39,9 | 39,9 | 39,1 |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%.

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[1] Discontinuation of exposure.

The mechanism of disturbance of the porphyrin metabolism during the action of toxic substances has been inadequately studied thus far. However, many believe that this disturbance takes place as a result of a depression of enzyme systems which is associated with a change in the cellular metabolism in the nervous system, liver, bone marrow, etc. (A. M. Chernyy, R. B. Mogilevskaya, and others).

The amount of coproporphyrin excreted with the urine during the action of toxic substances in low concentrations decreases in some cases (carbon monoxide, dowtherm, styrene, dimethylformamide), and increases in others (lead, pentene, toluylene diisocyanate).

The study of porphyrin metabolism in connection with the validation of the maximum permissible concentrations of chemical substances in atmospheric air has been widely adopted, partly because of the high sensitivity of this method (M. I. Gusev, V. A. Chizhikov, G. I. Solomin, D. G. Ododoshvili, and others).

The coproporphyrin excreted with the urine was determined spectrophotometrically. The content of coproporphyrins was obtained from the optical density of the maximum absorption at 402-403 mμ, determined with an SF-4 spectrophotometer. The urine was collected simultaneously from 5 rats of each group in the course of 24 hours, once a week. A total of 56 analyses were made, which amounted to 14 analyses in each experimental group of animals. The amount of coproporphyrin excreted with the urine by the rats, in micrograms per 100 g of weight, is shown in Table 3.

In animals of group III (0.15 mg/m$^3$), no statistically significant deviations in porphyrin metabolism were found as compared to the control. In group II (0.51 mg/m$^3$), a statistically significant decrease of the amount of coproporphyrin excreted with the urine was noted as early as the first three weeks since the start of exposure. This decrease gradually became more pronounced until the admission of acrolein to the chamber was discontinued.

In group I (1.52 mg/m$^3$), before the time when it was necessary to discontinue the exposure, the amount of excreted coproporphyrin had a tendency to increase, although this was found to be statistically insignificant. After the exposure was discontinued, the porphyrin metabolism began to decrease gradually, this decrease lasted for 5 weeks, and only in the 6th week of the recovery period did the excretion of coproporphyrin gradually level off, and in the 8th week reached the indices of the control group.

Table 3

Excretion of Coproporphyrin with the Urine in 24 Hours
(in micrograms per 100 g of weight).

| Period of Study From the Start of Exposure | Concentration of Acrolein, mg/m$^3$ | | | |
|---|---|---|---|---|
| | Control | 0.15 | 0.51 | 0.54 |
| First Three Weeks | 1,88 | 1,54 | 1,43 (a) | 1,93 |
| Second Three Weeks | 1,94 | 1,61 | 1,26 (b) | 0,99 (c)[1] |
| Third Three Weeks | 1,78 | 1,60 | 0,67 (c) | 0,72 (c) |
| Recovery Period | 1,87 | 1,61 | 1,50 | 1,59 |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%.

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[1] Starting on 24 April the exposure was discontinued.

Observations of the early physicochemical and structural changes taking place in the cells can be made by using the fluorescent microscopic method (M. N. Meysel' and A. V. Gutkina, 1953; M. N. Meysel' and V. A. Sondak, 1954; M. Ya. Khodas, 1954, and others). A. D. Semenenko (1963), who used the method of induced (secondary) fluorescence, observed changes in the leucocytes of blood taken from white rats in the course of chronic exposure to aniline in concentrations of 0.03, 0.3, and 3 mg/m$^3$.

In all three groups, a dependence was established between the manifestation of fluorescence and the concentration of the toxic substance, expressed in the degree of change of the cells and time of its manifestation.

M. I. Gusev and K. N. Chelikanov (1963) used the fluorescent method of study of blood leucocytes for an experimental validation of the mean daily maximum permissible concentration of pentenes in atmospheric air. A statistically significant increase in the number of fluorescent leucocytes was

Table 4

Number of Fluorescent Leucocytes (in percent).

| Group | Concentration of Acrolein, $mg/m^3$ | Before Exposure 22/III | Exposure | | | | | | Recovery Period | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 8/IV | 15/IV | 24/IV | 9/V | 19/V | 20/V | 11/VI | 19/VI |
| I | 1,52 | 3,95 | 21,70 (c) | 5,70 (a) | Exposure discontinued | — | 18,60 (b) | 3,70 | — | — |
| II | 0,51 | 4,40 | 12,40 (c) | 4,55 | 10,3 (b) | 15,80 (c) | 16,4 (b) | 9,50 (c) | 5,40 | 2,70 |
| III | 0,75 | 5,60 | 1,70 | 4,30 | 7,56 (a) | 9,60 (b) | 5,40 | 3,90 | 4,50 | 4,80 |
| IV | Control | 1,31 | 1,03 | 1,03 | 4,80 | 6,00 | 8,30 | 3,80 | 2,50 | 1,00 |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%.

established as compared with the control group of the animals. This increase occurred gradually, and the blood count returned to the original norm even during the period of exposure.

In our study, we determined the percent content of fluorescent leucocytes in the blood of rats during their exposure to acrolein. For this purpose, the blood taken was fluorochromed with acridine orange diluted 1:10,000, and examined under an ML-1 microscope. The results are shown in Table 4.

In animals of group I (1.52 $mg/m^3$), an increase in the percent content of fluorescent leucocytes of the blood took place in the very first week of exposure. Later it decreased somewhat, but still remained high as compared with the control. It should be pointed out that it was difficult to take the blood from rats of group I, because it coagulated rapidly. This explains the lack of data on the amount of fluorescent leucocytes on some days of the study. The content of fluorescent leucocytes remained high for 20 days after the forced discontinuation of the exposure, but after 27 days of the recovery period the percent content of fluorescent leucocytes returned to normal, so that further studies were discontinued.

In group II (0.51 $mg/m^3$), there were also statistically significant changes in the percent content of fluorescent leucocytes, which, starting with the first week of exposure, continued to remain high until the end of the exposure period. After 11 days of the recovery period, the number of fluorescent leucocytes began to decrease and reached the normal indices.

In animals of group III (0.15 $mg/m^3$), for which there were no changes in the other indices, a statistically significant increase in the number of fluorescent leucocytes of the blood as compared with the control group was observed 24 days after the start of exposure. Similar changes were also observed by other investigators. For example, A. D. Semenenko (1963) recorded significant changes in the blood of rats during their chronic exposure to aniline in a concentration of 0.03 mg/m$^3$, whereas P. G. Tkachev (1963), who used other methods of investigation, failed to observe any changes at this concentration. This makes it necessary to postulate that the method of fluorescent microscopy is very sensitive, so that it can be recommended for use in the establishment of maximum permissible concentrations of chemical substances in atmospheric air.

At the same time, it is necessary to study the nature of the process whereby the content of fluorescent leucocytes in the blood increases when the organism is acted upon by toxic substances in low concentrations.

After the chronic experiment was completed, some of the experimental animals were subjected to an anatomico-pathologic examination.* Histological examination of organs of animals in groups III and IV (0.15 mg/m$^3$ and control) failed to detect any marked differences. Changes were found mainly after the action of acrolein in concentrations of 0.51 and 1.52 mg/m$^3$ (groups I and II). Initial changes at the 0.51 mg/m$^3$ concentration were manifested in the proliferation of the columnar epithelium of the bronchi with hyperproduction of mucus and excessive infiltration of the bronchial walls by eosinophil leucocytes.

In rats exposed to acrolein in the 1.52 mg/m$^3$ concentration, there were marked changes of an inflammatory nature, in the form of purulent panbronchitis, bronchiolitis, and large-focus pneumonia (in one-half of the observations) in the respiratory organs, and complications of fibrous-purulent pleuritis in two cases. In the internal organs (myocardium, liver), marked dystrophic changes were observed in the form of granular and adipose dystrophy with small areas of necrobiosis.

## Conclusions

1. Prolonged inhalation of acrolein vapors in concentrations of 1.52-0.51 mg/m$^3$ by the experimental animals causes a weight loss, change of the conditioned reflex activity, decrease of cholinesterase activity, change of porphyrin metabolism in the direction of a decrease, and an increase in the percentage of fluorescent leucocytes of the blood.

2. Under the same conditions of exposure, acrolein in a concentration of 0.15 mg/m$^3$ has no effect on the organism of white rats with the exception of an increase in the number of fluorescent leucocytes of the blood. The method of induced (secondary) fluorescence permits a direct observation of the earliest physicochemical and structural changes in the cells, and can therefore

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* The anatomico-pathologic examinations were performed by K. L. Volchenko.

be recommended for use in the establishment of the mean daily maximum permissible concentrations of toxic substances in atmospheric air.

3. The mean daily maximum permissible concentration of acrolein in atmospheric air established at the present time and equal to 0.1 $mg/m^3$ is at the level of the subthreshold concentration and can be left unchanged.

## LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of this volume in the 1967 bibliography.

# STUDY OF THE REFLEX AND RESORPTIVE EFFECTS OF THIOPHENE

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 120-132, (1968).

Thiophene, a representative of a group of heterocyclic compounds, was discovered in 1883. From the standpoint of physical properties, it is a colorless liquid whose odor resembles that of benzene. In chemical properties, thiophene is stable toward oxidizing agents, does not enter readily into addition reactions, and is easily nitrated and sulfonated.

Thiophene is used in the production of plastics, antioxidants, dyestuffs, pharmaceutical preparations, insecticides, etc.

In its toxicological properties, thiophene is a nerve and blood poison (N. V. Lazarev, 1954; McCord, 1931; Hultgren, 1926; Christomanos, 1930; A. G. Mikhaylets and D. G. Pel'ts, 1964).

The extensive industrial use of thiophene, the possibility of its pollution of atmospheric air, and the lack of literature data on the toxicity of its low concentrations account for the relevance and timeliness of the present study.

To determine low thiophene concentrations, a spectrophotometric method was developed (Ye. P. Aigina and G. S. Terekhova), whereby the optical density of an alcohol solution of a thiophene mixture was measured at a wavelength of 231 mμ in quartz cells with a light path of 10 mm on an SF-4 instrument. The sensitivity of the method was 0.5 μg/ml, and the accuracy, 4-5%.

To study the reflex effect of low thiophene concentrations, we carried out physiological tests on people, widely employed in sanitary practice, which consisted in the determination of the threshold of olfactory perception, the light sensitivity of the eye (V. A. Ryazanov et al., 1957), and the bioelectric activity of the human cerebral cortex (K. A. Bushtuyeva, Ye. F. Polezhayev, and A. D. Semenenko, 1960). The resorptive action of thiophene was studied by means of physiological, hematological and biochemical tests such as the ratio of chronaxies of antagonist muscles, absolute number of leucocytes and the differential count, content of total protein and protein fractions of the blood serum, sulfhydryl groups and sialic acids in the blood serum by the method of Hess modified by N. N. Pushkina (1963), and coproporphyrin in the urine.

At the end of the exposure, we determined the ascorbic acid content in the organs and sulfhydryl groups and pyruvic acid in the liver by the Friedemann-Haugen method modified by Pushkina.

We began our experimental studies with the determination of the threshold of olfactory perception in 21 persons with a normal sense of smell. For two of the most sensitive persons of this group, the odor threshold of thiophene was found to be 2.1 mg/m$^3$, and the subthreshold concentration, 1.9 mg/m$^3$.

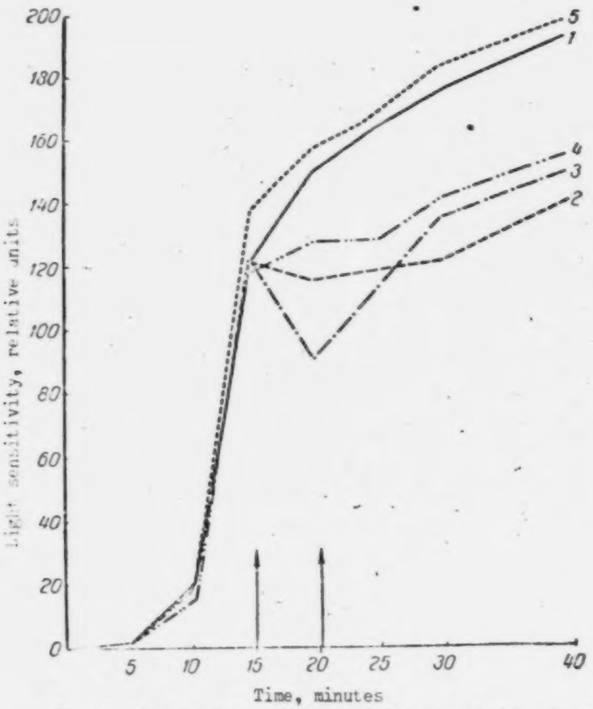

Fig. 1. Change in the light sensitivity of the eyes in subject M. during inhalation of thiophene vapors.
1 - pure air; 2 - concentration, 2.1 mg/m$^3$; 3 - 1.4 mg/m$^3$; 4 - 1.0 mg/m$^3$; 5 - 0.8 mg/m$^3$.

The reflex effect of thiophene concentrations of imperceptible odor was studied by using the dark adaptation method on three persons 19 to 40 years of age.

The effect of four thiophene concentrations (2.1, 1.4, 1.0 and 0.8 mg/m$^3$) supplied from the 15th through the 20th minute of the test was studied. The results of the experiment show that the first three thiophene concentrations had an effect on the light sensitivity of the subjects' eyes. In addition, in subject A. we noted an increase of the light sensitivity upon inhalation of thiophene vapors in concentrations of 2.1, 1.4 and 1.0 mg/m$^3$, with a high confidence factor of the changes obtained. In the two other persons we observed a decrease of the light sensitivity of the eye in response to the same concentrations (Fig. 1). The inhalation of thiophene vapors in a concentration of 0.8 mg/m$^3$ did not cause any substantial changes in the light sensitivity of the subjects' eyes. Data on the light sensitivity of the eyes during inhalation of thiophene are listed in Table 1.

Table 1

Change in the Light Sensitivity of the Eye in the Course of Dark Adaptation During Inhalation of Different Thiophene Concentrations (average values in percent of 15th minute).

| Subject | Period, Minutes | Pure Air | Thiophene Concentration, mg/m$^3$ | | | |
|---|---|---|---|---|---|---|
| | | | 2,1 | 1,4 | 1,0 | 0,8 |
| A. | 20 | 117,2 | 146,3 (b) | 157,4 (b) | 122,6 (o) | 126,6 (o) |
| | 25 | 124,5 | 211,6 (c) | 181,7 (b) | 142,2 (o) | 138,0 (o) |
| | 30 | 131,9 | 212,8 (c) | 197,0 (b) | 163,1 (a) | 146,7 (o) |
| | 40 | 147,1 | 263,1 (c) | 217,4 (b) | 179,4 (o) | 149,3 (o) |
| M. | 20 | 122,1 | 91,0 (a) | 74,8 (c) | 108,1 (a) | 117,3 (o) |
| | 25 | 133,2 | 97,2 (o) | 111,2 (o) | 112,9 (b) | 122,9 (o) |
| | 30 | 142,8 | 99,0 (a) | 99,1 (a) | 118,4 (a) | 135,4 (o) |
| | 40 | 156,5 | 115,0 (b) | 121,5 (o) | 129,6 (o) | 143,5 (o) |
| I. | 20 | 117,3 | 82,6 (c) | — | 97,5 (o) | 114,5 (o) |
| | 25 | 125,6 | 100,8 (c) | — | 103,9 (o) | 120,2 (o) |
| | 30 | 135,1 | 109,0 (a) | — | 114,8 (b) | 131,2 (o) |
| | 40 | 143,7 | 124,2 (b) | — | 124,4 (b) | 139,5 (o) |

Note. Confidence factor: a - 95%, b - 99%, c - 99.9%, o - unreliable.

In the next stage of our studies we investigated the biopotentials of the cerebral cortex in three persons with the aid of quantitative analysis of the reflex response of the alpha-rhythm burst (A. D. Semenenko et al., 1963). The method is based on the reinforcement of the intrinsic potentials of the brain under the influence of different rhythmic stimuli.

The study was made on an eight-channel electroencephalograph of the "Orion Budapest" Co. The biocurrents were recorded from the temporal-occipital regions.

It is known that the alpha rhythm in man is variable, and therefore in order to detect fluctuations in the rhythm of the brain under the influence of low thiophene concentrations, we attempted to develop in the subjects a dynamic stereotype to the conditions of the experiment.

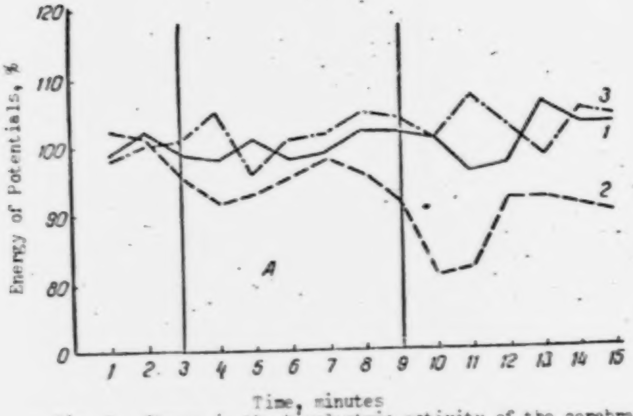

Fig. 2. Change in the bioelectric activity of the cerebral cortex in subject L. during inhalation of thiophene vapors (left hemisphere).
1 - pure air; 2 - concentration, 0.8 $mg/m^3$; 3 - 0.6 $mg/m^3$.

During the test, the subjects were exposed to a rhythmic light of different intensities (18 seconds), which was followed by a light muscular workout (25 seconds), a weak sound of variable frequency (10 seconds) and expectation of the next light stimulus (7 seconds); these periods were repeated 15-18 times in each observation.

Changes of the brain biopotentials during inhalation of thiophene vapors in a concentration of 0.8 $mg/m^3$ were analyzed by means of an automatic integrator and were characterized by both a reinforcement and a depression of the biopotentials. Statistical treatment of the data confirmed the significance of the changes, compared with the results obtained after the inhalation of pure air.

We observed a reinforcement of the brain's biopotentials in subjects Yu. and C., and a depression in L. (Fig. 2, Table 2).

Thiophene in a concentration of 0.6 $mg/m^3$ had no significant effect on the brain biopotentials of the subjects. Since this value is shown to be the subthreshold concentration by the most sensitive test, we propose it as the highest single maximum permissible concentration in atmospheric air.

Combined data from the study of the reflex effect are listed in Table 3.

Table 2

Change in the Energy of the Brain's Biopotentials During Inhalation of Different Thiophene Concentrations (average values in percent of the first three minutes - background period).

| Subject | Period, minutes | Right Hemisphere | | | Left Hemisphere | | |
|---|---|---|---|---|---|---|---|
| | | Pure Air | 0,8 mg/m$^3$ | 0,6 mg/m$^3$ | Pure Air | 0,8 mg/m$^3$ | 0 6 mg/m$^3$ |
| L. | 4—5 | 100,4 | 100,8 (o) | 100,0 | 98,2 | 93,3 | 100,6 (o) |
| | 6—7 | 101,0 | 104,8 | 99,6 | 98,4 | 98,2 | 101,8 (o) |
| | 8—9 | 102,4 | 103,1 | 103,2 | 102,1 | 93,8 (a) | 104,7 (o) |
| | 10—11 | 99,8 | 90,4 | 102,8 | 98,7 | 81,1 (a) | 104,0 (o) |
| | 12—13 | 100,7 | 103,2 | 99,2 | 101,4 | 92,2 | 100,5 (o) |
| | 14—15 | 102,8 | 102,5 | 100,8 | 103,0 | 90,8 (b) | 104,7 (o) |
| Yu. | 4—5 | 98,2 | 97,3 | 101,8 | 101,1 | 109,6 (a) | 100,7 (o) |
| | 6—7 | 96,8 | 100,5 | 96,4 | 104,1 | 109,6 | 93,4 (o) |
| | 8—9 | 99,0 | 99,2 | 103,2 | 105,8 | 116,9 (a) | 98,8 (o) |
| | 10—11 | 99,6 | 94,3 | 101,8 | 104,3 | 119,0 (b) | 101,8 (o) |
| | 12—13 | 97,2 | 95,9 | 100,2 | 103,5 | 111,3 | 99,1 (o) |
| | 14—15 | 99,2 | 101,2 | 103,5 | 100,6 | 113,5 (a) | 103,5 (o) |
| G. | 4—5 | 103,4 | 109,6 | 97,7 | 104,9 | 99,2 | 100,8 |
| | 6—7 | 105,0 | 115,8 (a) | 101,8 | 107,2 | 100,3 (a) | 103,7 |
| | 8—9 | 102,1 | 107,7 | 103,2 | 105,6 | 101,3 | 98,2 |
| | 10—11 | 102,9 | 111,6 | 103,2 | 105,7 | 98,0 | 101,2 |
| | 12—13 | 105,9 | 110,0 | 105,2 | 111,1 | 101,5 (a) | 102,8 |
| | 14—15 | 111,6 | 107,4 | 104,1 | 109,5 | 102,2 | 96,4 |

Note. Confidence factor: a - 95%; b - 99%; o - unreliable.

Table 3

Reflex Effect of Thiophene on the Human Organism.

| Reflex Effect Index | Thiophene Concentration, mg/m$^3$ | |
|---|---|---|
| | Threshold | Subthreshold |
| Olfactory Perception . . . . . . . | 2,1 | 1,9 |
| Light Sensitivity of the eye . . . . | 1,0 | 0,8 |
| Bioelectric activity of the cerebral cortex . . . | 0,8 | 0,6 |

We studied the resorptive effects of thiophene in the course of round-the-clock chronic exposure on 60 white male rats during 80 days. The animals were divided into four groups: the first was exposed to a thiophene concentration of 20 mg/m$^3$, the second to 3 mg/m$^3$, the third to 0.6 mg/m$^3$, and the fourth group was the control. During the experiment, observations were made every 15 days; the results were treated statistically.

The general state of the animals during the course of the experiment remained unchanged. The rats were active and gained weight normally. The results of treatment of the weight of the rats in percent of the initial weight remained unreliable compared with the control.

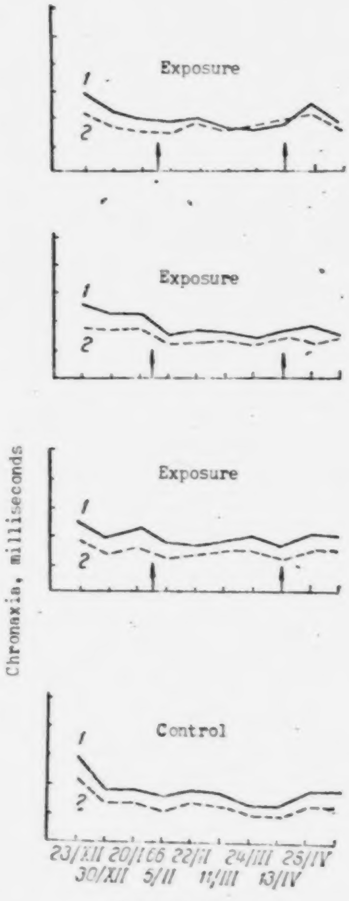

Fig. 3. Disturbance of the motor chronaxy of antagonist muscles in experimental rats during exposure to thiophene vapors.

1 - extensors; 2 - flexors.

In the study of the chronaxy of extensors and flexors during the experiment, statistically reliable changes expressed in a convergence of the chronaxies of flexors and extensors were observed in rats of the first group (20 mg/m$^3$). Further studies showed an inversion of the ratios of chronaxies of flexors and extensors in rats of the first group, and these changes occurred before other indices, in the third week of the experiment (Fig. 3). A restoration of the normal ratio of chronaxies of antagonist muscles in this group of rats occurred on the 26th day after the end of exposure. We observed shifts in the leucocyte content later than changes in the chronaxies of antagonist muscles. Leucopenia occurred in rats of the first group (20 mg/m$^3$) as late as the 6th week of exposure, and lymphopenia, in the 8th. The restoration of leucocytes also occurred on the 26th day, and that of lymphocytes, on the 10th day after the end of the experiment (Fig. 4).

The total amount of blood serum proteins in rats of all the groups did not show any changes in the course of the experiment. Deviations in the protein fractions which occurred in the fourth week were observed only in rats of the first group.

These changes were characterized by a decrease in the amount of albumin, an increase of gamma globulin, and a decrease of the albumin-globulin ratio, this being in agreement with the data of a number of authors who found changes in the fractional composition of the blood serum of people and animals under the influence of harmful chemical agents (V. A. Chizhikov, 1964; P. G. Tkachev, 1964; K. A. Bushtuyeva, 1966). Further studies of the relative amounts of protein fractions in rats of the first group revealed changes in the beta globulin fraction which took place in the sixth week of the experiment and returned to normal at the end of the experiment. Changes in the relative amounts of the protein fractions in rats of the first group were confirmed by results of histopathological analyses. It is probable that the shift which we observed in the relative amounts of protein fractions in rats were due to the toxic effect of thiophene on the liver.

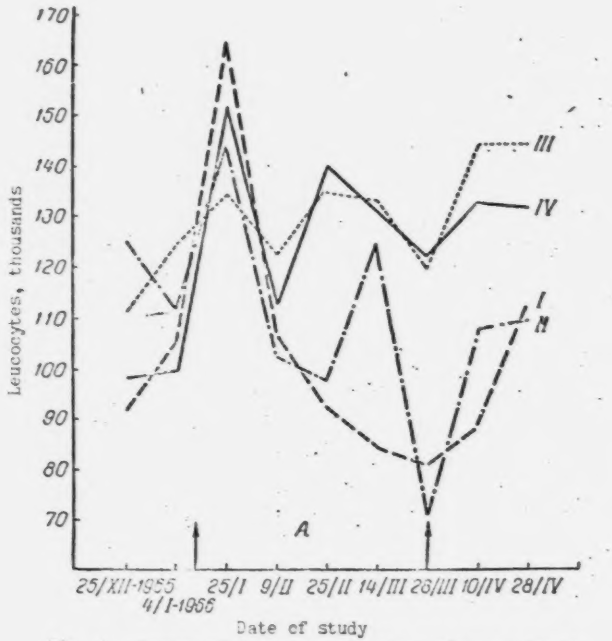

Fig. 4. Number of leucocytes in the peripheral blood of rats of different groups.
Group I - 20 mg/m$^3$; Group II - 3 mg/m$^3$; Group III - 0.6 mg/m$^3$; Group IV - control.

The content of sulfhydryl groups in the blood serum of rats of the first group decreased in the fourth week. We attribute these changes to functional shifts in the course of the processes of inhibition and stimulation of the nervous system, brought about by the action of thiophene.

A reliable increase in the content of sialic acids in the blood serum of rats of the first group was detected at the end of the experiment.

In the third week of the experiment, a decrease in the excretion of coproporphyrin with the urine was established (Fig. 5), which may be regarded as a consequence of a disturbance of the activity of the central nervous system (Yu. K. Smirnov, 1957). More pronounced changes in the excretion of coproporphyrin with the urine of rats were observed during the action of thiophene in a concentration of 20 mg/m$^3$ than at 3 mg/m$^3$, and these changes lasted a long time after the exposure.

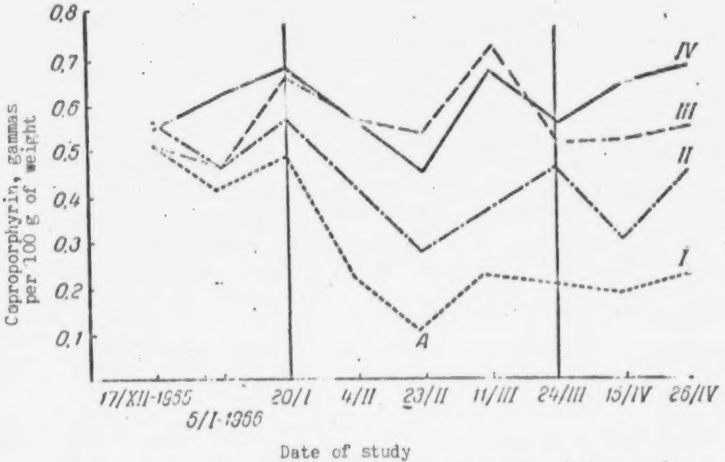

Fig. 5. Dynamics of excretion of coproporphyrin with rat urine during exposure to thiopene vapors. Notation same as in Fig. 4.

One of the biochemical indicators of vitamin metabolism was the determination of ascorbic acid in the organs of the rats at the end of the experiment. A disturbance of vitamin C metabolism indicates an unfavorable influence of atmospheric pollutants (Ye. F. Yelfimova, and N. N. Pushkina; V. A. Yakamees, 1966). We established a significant decrease in the content of ascorbic acid in the brain, liver, kidneys and adrenal glands of animals of the first group. The most pronounced changes were observed in the kidneys.

A decline in the amount of ascorbic acid, a decrease of the sulfhydryl groups and an increase of pyruvic acid in the liver of animals of this group correlated with histopathological data indicating a decrease of glycogen.

In rats of the second group (3 mg/m$^3$), the changes developed more slowly. Shifts in the ratio of chronaxies of antagonist muscles occurred as late as the sixth week of the experiment. Leucopenia in the peripheral blood of rats of this group was observed as late as the sixth week and was unstable. No change was noted in the number of lymphocytes. The concentration of the protein fractions of the blood serum in rats of the second group remained without any appreciable changes over the course of the experiment, with the exception of the gamma globulin fraction, which increased in the fourth week and gave significant results by statistical treatment. These changes may be regarded as a defensive-adaptive reaction of the body to the influence of a harmful agent, in particular, thiophene (3 mg/m$^3$). The increase in the content of gamma globulin may also be due to the activation of Kupffer's cells and plasma cells in the organism of this group of rats (histopathological data).

The manifestation of the toxic effect of thiophene at the 3 mg/m$^3$ concentration on sulfhydryl groups of the blood serum of rats was confirmed statistically and occurred later than in rats of the first group, i. e., in the sixth week of the experiment.

A change in the amount of coproporphyrin in the urine was also observed in this group of rats, but to a lesser extent and later than in rats of the first group.

Table 4

Results of the Resorptive Effect of Thiophene on the Organism of White Rats.

| Indicator | Thiophene Concentration, mg/m$^3$ | | | |
|---|---|---|---|---|
| | 20 | 3,0 | 0,6 | Control |
| Weight of animals | — | — | — | — |
| Ratio of chronaxies of antagonist muscles | + | + | — | — |
| Total protein | — | — | — | — |
| Protein fractions: | | | | |
| albumin | + | — | — | — |
| globulins: | | | | |
| α | — | | | |
| β | + | | | |
| γ | + | | | |
| Albumin-globulin ratio | + | — | — | — |
| Leucocytes (leucopenia) | + | + | — | — |
| Lymphopenia | + | — | — | — |
| Coproporphyrin in urine | + | + | — | — |
| Sulfhydryl groups in blood serum | + | + | — | — |
| Ascorbic acid: | | | | |
| in brain | + | + | — | — |
| in liver | — | + | — | — |
| in kidneys | + | + | — | — |
| in adrenal glands | + | + | — | — |
| Sulfhydryl groups and pyruvic acid of liver | + | — | — | — |
| Sialic acids in blood serum | + | — | — | — |

Symbols: + reliable result, - unreliable result.

The ascorbic acid content of the internal organs of rats also underwent a reliable decrease. Changes in pyruvic acid, sulfhydryl groups in the liver and sialic acids in the blood serum were not reliable.

In the third group (thiophene concentration, 0.6 mg/m$^3$) the results of the studies differed little from the control and were not statistically reliable. Combined data on the resorptive effect of thiophene on the experimental rats are shown in Table 4.

Functional changes in rats of the first and second groups were confirmed by histopathological studies, which showed the presence in the lungs of neutrophils, areas of desquamative bronchial epithelium, and proliferation of the epithelium of the bronchi in the lungs. In the liver, an irregular engorgement of the capillary network was noted, activated forms of Kupffer's cells were frequently found in the lumen of the sinuses (round macrophages, and accumulation of neutrophils in the lumen of large vessels), and lymphoid infiltration appeared around the triads. There was a distinct decrease of glycogen over the periphery of hepatic lobules. No such changes were found in rats of the third group.

## Conclusions

1. The threshold concentration for olfactory perception of thiophene is 2.1 mg/m$^3$, and the subthreshold concentration, 1.9 mg/m$^3$.

2. The thiophene concentration acting on the light sensitivity of the eyes is below the threshold of olfactory perception and equal to 1 mg/m$^3$, and the inactive concentration is 0.8 mg/m$^3$.

3. Changes in the bioelectric activity of the cerebral cortex of the subject were observed during inhalation of thiophene vapors in a concentration of 0.8 mg/m$^3$; a concentration of 0.6 mg/m$^3$ was found to be inactive.

4. A prolonged round-the-clock action of thiophene in concentrations of 20 and 3 mg/m$^3$ gives rise to a series of physiological, hematological, biochemical and pathomorphological changes in the body of the experimental animals. The 0.6 mg/m$^3$ thiophene concentration was found to be inactive.

5. The highest single and mean daily concentrations of thiophene which can be recommended are at a level of 0.6 mg/m$^3$.

## LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

# SANITARY-TOXICOLOGICAL APPRAISAL OF THE COMBINED EFFECT OF A MIXTURE OF BENZENE AND ACETOPHENONE VAPORS IN ATMOSPHERIC AIR

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 11, Izdatel'stvo "Meditsina" Moskva, p. 107-120, (1968).

The toxic effect of benzene at and slightly above the level of the maximum permissible concentration for plant shops was studied by A. P. Volkova (1960), Gabor and Raucher (1960), A. I. Korbakova et al. (1965), and others. The toxic effect of low concentrations of these substances was investigated by Yu. V. Novikov (1956) and I. S. Gusev (1965).

In analyzing the data obtained by the different authors one can conclude that benzene in low concentrations has a predominant influence on the central nervous system and causes a series of changes in the blood-forming system, primarily in the white blood cells. A decrease in the number of thrombocytes in the blood is considered to be one of the early signs of benzene poisoning.

In a study of the effect of large concentrations of benzene in combination with chloroform and toluene, T. A. Shtessel' (1938) observed a simple summation effect of their action. During the combined action of large concentrations of benzene and acetone, M. I. Ol'shanskiy and V. V. Likhacheva (1935) noted an enhancement of the influence of the mixture. A prolonged combined action of benzene and acetone in concentrations at and above the level of those in plant shops was studied by N. A. Zhilova (1959). On the basis of the experiments performed, the author holds that the benzene-acetone mixture irritates the central nervous system and blood-forming organs much more strongly than any of these substances does individually.

The effect of low concentrations of acetophenone, both during short-term exposure of man and under conditions of a chronic experiment on animals, was studied by N. B. Imasheva (1963), who observed marked functional changes in the central nervous system and in the proportion of protein fractions of the animal blood. During the combined action of low concentrations of acetophenone with phenol (Yu. Ye. Korneyev, 1965) and acetophenone (N. Z. Tkach, 1965), the authors found a simple summation of the effects.

The literature contains no studies dealing with the combined action of low concentrations of benzene and acetophenone discharged into atmospheric air during the production of synthetic phenol and acetone.

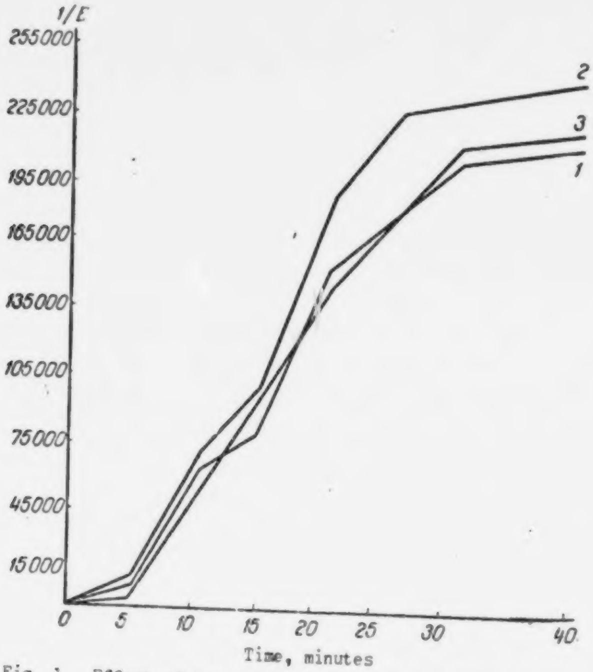


Fig. 1. Effect of low concentrations of a mixture of benzene and acetophenone vapors on the light sensitivity of the eye in subject Sh. Yu.
1 - pure air; 2 - gaseous mixture with a total concentration index of 1.03; 3 - gaseous mixture with a total concentration index of 0.75.

The object of the present paper was to study the nature of the biological effects of a mixture of low benzene and acetophenone concentrations in atmospheric air.

The character of the inhalational effect of this mixture on man was evaluated by determining the thresholds of its olfactory perception and its reflex effect on the light sensitivity of the eye and bioelectric activity of the brain. These tests are based on the influence exerted on the bodily functions by an extraneous stimulation focus arising in the central nervous system during inhalation of the substances studied.

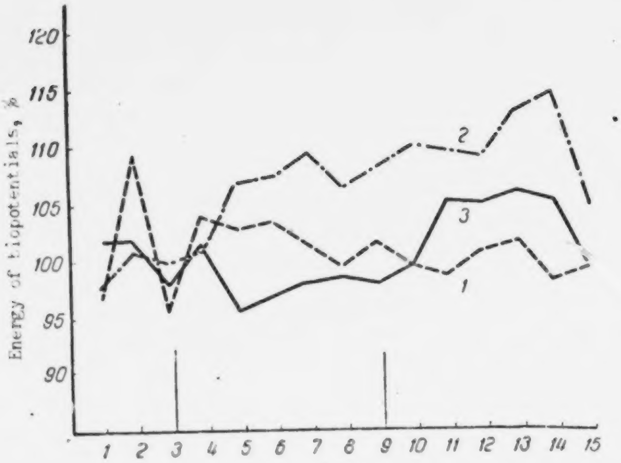


Fig. 2. Change in the bioelectric activity of the brain in subject R. L. during inhalation of a mixture of benzene and acetophenone vapors. Left hemisphere.
1 - pure air; 2 - gaseous mixture with a total concentration index of 1.05; 3 - gaseous mixture with a total concentration index of 0.88.

Table 1

Thresholds of the Reflex Effect for the Most Sensitive Persons During the Combined Action of Benzene and Acetophenone.

| Olfactory Perception | | | | Light Sensitivity of the Eye | | | | Bioelectric Activity of the Brain | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Minimum perceptible concentration mg/m³ | Total concentration index | Maximum imperceptible concentration, mg/m³ | Total concentration index | Threshold concentration, mg/m³ | Total concentration index | Subthreshold concentration, mg/m³ | Total concentration index | Threshold concentration, mg/m³ | Total concentration index | Subthreshold concentration, mg/m³ | Total concentration index |
| $\frac{1.46}{0{,}0032}$ | 1 | $\frac{1{,}18}{0{,}0039}$ | 0,79 | $\frac{1{,}2}{0{,}005}$ | 1 | $\frac{0{,}86}{0{,}0035}$ | 0,75 | $\frac{1{,}1}{0{,}0035}$ | 1,05 | $\frac{0.9}{0{,}003}$ | 0,88 |

Note. Numerator of the fraction - benzene, denominator - acetophenone.

As is evident from Table 1 and also Figs. 1 and 2, all the tests showed the active mixture of benzene and acetophenone vapors to be one whose total concentration index in fractions of the threshold values for isolated action of the ingredients was equal to unity; for the inactive mixture, this index was below unity, i. e., the effect takes place in accordance with a simple summation.

The most sensitive was found to be the electroencephalographic test (minimum active mixture of benzene and acetophenone vapors in concentrations of 1.1 and 0.0035 mg/m$^3$ respectively).

In expressing the total content of the mixture employed in fractions of the maximum permissible concentration of each component, it was found that mixture I (minimal active) consists of:

$$\frac{1.1}{1.5}\text{ benzene} + \frac{0.0035}{0.0030}\text{ acetophenone} = 1.89,$$

and mixture II (maximum inactive), of

$$\frac{0.9}{1.5}\text{ benzene} + \frac{0.003}{0.003}\text{ acetophenone} = 1.6.$$

The studies performed lead to the conclusion that when benzene and acetophenone are simultaneously present in atmospheric air, their total concentration in fractions of the maximum permissible concentration of the substances for isolated action should not exceed 1.5.

Whereas the highest single maximum permissible concentrations are designed to prevent a degree of atmospheric pollution which could cause reflex responses through the irritation of receptors of the respiratory organs, the mean daily standards prevent a chronic resorptive effect of toxic substances during prolonged inhalation.

During the action of very low concentrations of toxic substances under conditions of a chronic experiment, the functional changes are basically nonspecific in character and should be regarded as defensive-adaptive responses (V. A. Ryazanov, 1964).

In order to evaluate the toxic effect of a mixture of benzene and acetophenone vapors on the animal organism, the following factors were observed: general state, behavior and dynamics of the weight of the animals, change of the motor chronaxy of antagonist muscles, content of leucocytes, erythrocytes and thrombocytes in the blood, differential white count, total content of nucleic acids in the blood, and amount of 17-ketosteroids in the urine. At the end of the experiment, some of the animals were killed and subjected to anatomicopathological and histological analyses.

The inhalational exposure of 45 white male rats was carried out continuously for 84 days. The rats were divided into three groups of 15 each.

The first group of animals was subjected to the action of a mixture of benzene and acetophenone vapors in a concentration 10 times the maximum inactive mixture of substances used in the study of the reflex effect on the biocurrents of the brain. On the average, the mixture consisted of 9 ± 0.194 mg/m$^3$ benzene and 0.030 ± 0.0033 mg/m$^3$ acetophenone.

The second group of animals inhaled a mixture at the level of the highest inactive concentration in the electroencephalographic tests. On the average, it contained 0.9 ± 0.2 mg/m$^3$ benzene and 0.003 ± 0.00005 mg/m$^3$ acetophenone.

The third group of animals served as the control.

To evaluate the functional state of the cortex of the large hemispheres, the ratio of chronaxies of antagonist muscles was studied, whose change is affected by central influences.

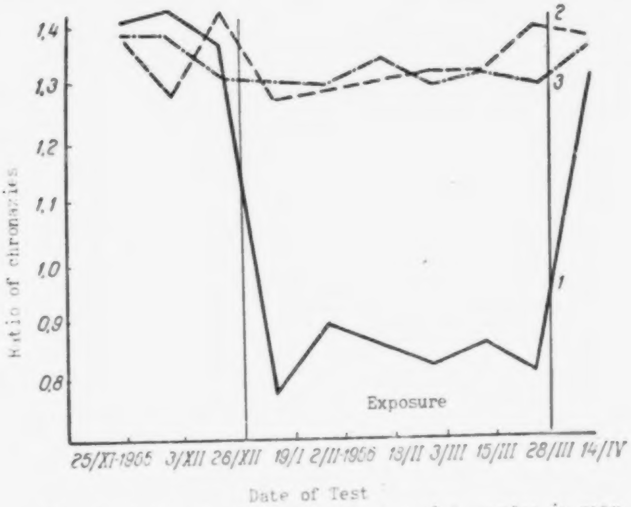


Fig. 3. Ratio of chronaxies of antagonist muscles in rats exposed to benzene and acetophenone vapors.
1 - first group (total concentration index 9.0); 2 - second group (total concentration index 0.9); 3 - third group (control).

The motor chronaxy of antagonist muscles (extensors and flexors of the shin) was studied in five rats of each group once every 15 days with the aid of an ISE-01 electronic pulse stimulator.

The results of the tests, conducted in accordance with a standard procedure, are shown in Fig. 3, from which it is apparent that a change in the ratio of chronaxies of antagonist muscles manifested itself very early in the first group of animals, on the 15th day of exposure, and remained below unity during the entire period of exposure. This indicates a substantial effect of mixture I, which caused functional changes in the central nervous system and was manifested in a decrease of the subordinating influence of the center on the ratio of chronaxies of antagonist muscles.

Considering the literature data on the specific effect of benzene on the white blood cells, we studied the influence of this mixture of vapors on the absolute content of leucocytes, the differential count, and the thrombocyte content. As was shown by the results, the leucocyte content dropped considerably in the first group, but the changes reached a statistically reliable difference, compared with the control only in the third month of exposure (Fig. 4).

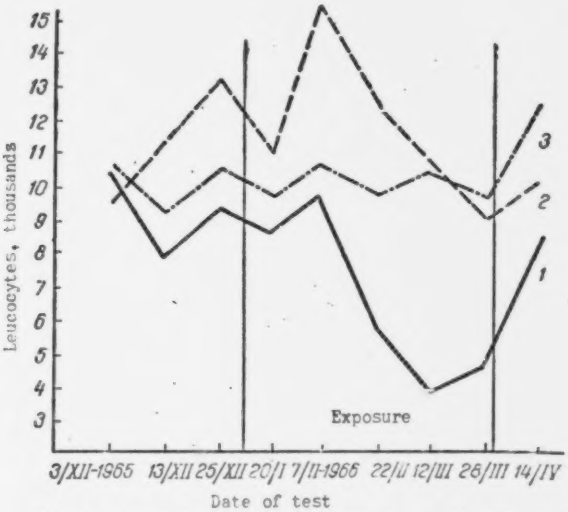


Fig. 4. Average content of leucocytes in the blood of rats exposed to benzene and acetophenone vapors. Notation same as in Fig. 3.

In the differential count, a slight shift to the left (increase in the number of staff cells) and an appreciable decrease in the absolute number of lymphocytes were observed at that time.

Toward the end of the second month of exposure, the content of thrombocytes in the blood of rats of the first group dropped sharply (Fig. 5). No appreciable changes were found in the erythrocyte content.

As is evident from the results of the studies, changes in the peripheral blood occurred much later than in the central nervous system. It may be postulated that this system, being the most sensitive to the action of a mixture of two substances with a narcotic effect, played the major role in the change of the blood composition.

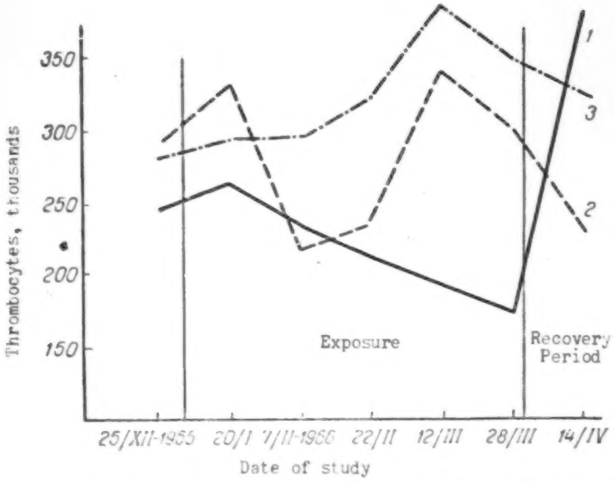


Fig. 5. Average thrombocyte content in the blood of rats of different groups. Notation same as in Fig. 3.

At the present time, a considerable amount of data has accumulated in support of an important role of nucleic acids in various processes connected with the manifestation of life and primarily with protein synthesis and the growth and division of cells.

A series of physiological states of the organism are related to the intensification of the synthesis and metabolism of nucleic acids. Particularly intense is the nucleic metabolism in processes of blood formation, enzyme synthesis, and formation of protein secretions (S. S. Debov, 1954).

Many toxic substances acting on the body lead to a quantitative change in the content of nucleic acids in certain organs and media (Campbell and Kosterlitz, 1952; Rambach, Moomav, Aet, and Cooper, 1952, and others).

A major part in the vital activity of the organism is played by the defensive function of nucleic acid.

A. I. Oparin and T. N. Yevreinova (1947) showed that when RNA is added to various proteins, their thermostability increases.

A. N. Belozerskiy (1944) held that the defensive function of DNA is manifested in the fact that it strongly binds alkaline proteins of the type of histones or protamines. It should be added that by forming such compounds, a nucleic acid not only neutralizes alkaline proteins, but also strips them of their toxic properties.

Ehrich, Drabkin and Forman (1949) showed a relationship between nucleic acids and the production of antibodies by mature plasma cells of lymphatic nodes. Goret (1949) pointed out that the nucleoproteins of cells destroyed in the body are a factor stimulating leucocytosis and phagocytosis.

To determine the total content of nucleic acids in the blood, we used a method proposed by P. V. Simakov (1960). The recipe was based on the general principles of the method of determination of nucleic acids in biological substances, developed by A. S. Spirin (1958) and applied to blood.

The method is based on the extraction of nucleic acids with hot perchloric acid, followed by the determination of nucleic phosphorus in the solution by photometry at wavelengths of 270 and 290 mμ; the content of nucleic acids is calculated from the concentration of nucleic phosphorus.

The determination was made in five rats (separately) of each group.

As is evident from Fig. 6, the content of nucleic acids in the blood of rats of the first group increased substantially relative to the control as early as the end of the first month of exposure and remained high until the end of the period of exposure. The amount of these acids in the blood of rats of the second group differed insignificantly from the data for the control. On the 17th day of the recovery period, the content of nucleic acids in the blood of rats of the first group decreased to the level of the control.

The increase in the amount of nucleic acids in the blood of rats of the first group was apparently due to an increase in the content of staff cells in the blood, reflecting an intensification of the defensive function of the organism in response to the action of the irritant.

It is well known that the penetration of young cells into the blood causes a severalfold increase in the content of nucleic acids (Rurt, Murray and Rossiter, 1951).

Thus, changes in the quantitative content of nucleic acids in the blood were found to be a sensitive indicator of the functional shifts occurring in the animal body under conditions of chronic exposure to microdoses of benzene in combination with acetophenone.

Studies have shown (Yu. Ye. Korneyev, 1965; N. Z. Tkach, 1965) that the determination of 17-ketosteroids in the urine is a sensitive test for evaluating the nonspecific effect of toxic substances on the functional state of the hypophyseal - adrenocortical system under the conditions of a chronic experiment.

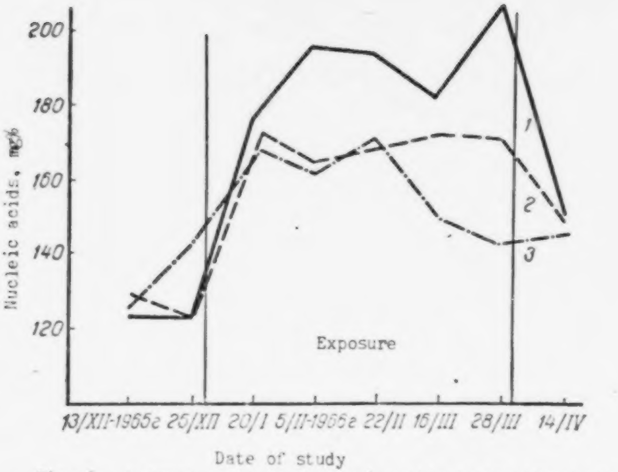


Fig. 6. Average content of nucleic acids in the blood of rats exposed to benzene and acetophenone vapors. Notation same as in Fig. 3.

The analysis was carried out on the total urine of five rats of each group.

Our studies showed that the content of 17-ketosteroids in the urine of animals of the first group was higher than normal during almost the entire course of exposure, but no significant difference in the results was obtained relative to the control.

It should be noted that after the completion of exposure, all the changes detected in the body of the experimental animals returned to normal in a comparatively short period of time (16-18 days). Hence, the shifts observed were relatively slight and functional in character.

No changes could be observed in animals of the second group, which inhaled a mixture of $0.9 \pm 0.02$ mg/m$^3$ benzene and $0.03 \pm 0.00005$ mg/m$^3$ acetophenone.

The internal organs and brain of the rats were subjected to histopathological analysis. In rats of the first group, a slight focal proliferation of structures of the epithelium lining the bronchi was observed, apparently formed in response to irritation by the inhaled vapors of benzene and acetophenone. The parenchyma of the liver had dilated sinuses. The nuclei of the liver cells retained their structure and only isolated ones were pyknotic. Kupffery cells were markedly hypertrophied, indicating activation of the reticuloendothelial system of the liver.

Some metabolic processes of the liver were studied by means of histochemical reactions. A marked decrease in the content of glycogen in the cells was observed, indicating a certain decline in the functional activity of the liver. In the determination of the content of RNA in the liver cells, an intensive synthesis of which indicates a high biological activity of the organ, a certain decrease of the pyroninophilic granules in the cytoplasm of the liver cells was noted. The RNA granules were often irregularly distributed, and the intensity of this reaction showed slight fluctuations in certain animals.

In the cerebral cortex in animals of the first group, many nerve cells were in a state of segmental chromatolysis. The chromatolysis observed in the neurons of the cerebral cortex was reversible; this was demonstrated by merely discontinuing the action of benzene and acetophenone vapors on the body, whereupon the chromatolysis effects disappeared.

A study of the reaction for RNA in the nerve cells of the cerebral cortex showed that the effect of a mixture of benzene (9 mg/m$^3$) and acetophenone (0.003 mg/m$^3$) on the body leads to a decrease of RNA, compared with the control.

The following conclusion may be drawn from the results of the chronic experiment. The inhalation of mixture I of substances by the animals caused marked changes in the functional state of the central nervous system, manifested in a change of the normal ratio of chronaxies of the antagonist muscles. The changes detected were confirmed by studying the state of the nerve cells of the cerebral cortex.

A normal ratio of chronaxies of antagonist muscles was observed during the recovery period. Changes in the nerve cells of the cerebral cortex were also reversible. Consequently, changes in the central nervous system were only functional in character.

Much later than in the central nervous system, in the course of the experiment, changes were detected in the peripheral blood (first group of rats), manifested in leucopenia, absolute lymphopenia, a slight shift of the differential count to the left, and thrombocytopenia.

Another highly sensitive test was found to be the determination of the total content of nucleic acid in the blood, whose amount during the exposure was increased in rats of the first group, compared with the control.

No reliable change in the number of erythrocytes in the blood and 17-ketosteroids in the urine could be obtained.

The changes detected in rats of the first group during the exposure returned to normal during the recovery period. In rats of the second group, no reliable changes relative to the control were observed.

Thus, mixture II of the substances was found to be inactive toward the organism of rats during a prolonged, continuous inhalation.

In order to study the pollution of atmospheric air with benzene and acetophenone vapors, analyses were carried out around a typical industrial plant producing synthetic phenol and acetone; they showed that at a distance of 100 m from the source of discharge, the highest single total concentration of the mixture is 197 times the recommended norm, and at distances of 300 and 500 m, 21.8 and 6.28 times respectively (Table 2). At a distance of 1000 m, benzene and acetophenone were not detected in the atmosphere. Yu. Ye. Korneyev, U. G. Pogosyan and N. Z. Tkach (1965) also failed to detect any pollution of air with phenol and acetone at this distance.

Table 2

Pollution of Atmospheric Air with Benzene and Acetophenone (in milligrams per $m^3$) Around a Synthetic Phenol and Acetone Plant.

| Substance | Distance From Source of Discharge, m | Number of Samples | Limits of Fluctuations | Average Concentration |
|---|---|---|---|---|
| Benzene | 100 | 16 | 2,1—4,73 | 3,3 |
| | 300 | 26 | 0,030—1,18 | 0,68 |
| | 500 | 25 | 0,010—0,18 | 0,091 |
| | 1000 | 25 | Negative Results | |
| Acetophenone | 100 | 11 | 0,050—0,879 | 0,258 |
| | 300 | 17 | 0,032—0,0967 | 0,0575 |
| | 500 | 26 | 0,0030—0,0286 | 0,0076 |
| | 1000 | 25 | Negative Results | |
| Total concentration above proposed norm (1.5) | 100 | 197 times greater | | |
| | 300 | 21.8 " " | | |
| | 500 | 6.28 " " | | |

It may be assumed that for the plant studied, the width of the sanitary protective zone with respect to such leading components of atmospheric discharge as benzene, phenol, acetone and acetophenone should be no less than 1 km.

## Conclusions

1. In a study of the reflex effect of a mixture of benzene and acetophenone vapors on the human body, a complete summation of the effects of their action was found; the total concentration index of the threshold mixtures is equal to 1.

2. Chronic round-the-clock exposure of rats for 84 days to a mixture of benzene and acetophenone vapors in concentrations of 9.0 and 0.030 mg/m$^3$ causes a series of statistically reliable functional shifts in the body of the animals: in the central nervous system, blood system, and in the total content of nucleic acids in the blood.

Pathomorphological changes of reversible nature in certain internal organs were also observed.

3. Chronic exposure under the same conditions to a mixture of benzene and acetophenone vapors in concentrations at the level of the maximum inactive ones as determined by the electroencephalographic test failed to cause any reliable shifts in the animal organism, compared with the control.

4. When benzene and acetophenone vapors are present together in atmospheric air, the total concentration of the mixture in fractions of the maximum permissible concentration of each of the substances should not exceed 1.5.

5. The sanitary protective zone between the boundary of synthetic phenol and acetone plants and the boundary of the residential area should be at least 1 km wide.

## LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

was made according to a procedure recommended by the Committee on Sanitary Protection of Atmospheric Air. First, all the subjects were familiarized with the odors of phenol and acetophenone, then the thresholds of olfactory perception of each of these substances were checked. A total of 22 persons participated in these experiments. For two subjects, the odor threshold concentration of phenol was 0.017 mg/m$^3$ (subthreshold concentration, 0.016 mg/m$^3$); 14 subjects identified the minimum phenol concentration at a level of 0.022 mg/m$^3$ (subthreshold concentration 0.017 mg/m$^3$); in the remaining subjects, the odor threshold was at higher levels. B. Mukhitov established the threshold of olfactory perception of phenol for the most sensitive persons at the level of 0.022 mg/m$^3$.

The odor threshold of acetophenone for 10 subjects was found to be 0.010 mg/m$^3$ (subliminal concentration 0.008 mg/m$^3$). For 11 persons, the threshold concentration was at a level of 0.0285 mg/m$^3$ (subliminal concentration of 0.01 mg/m$^3$). The odor threshold for one subject was above 0.03 mg/m$^3$. The threshold of olfactory perception of acetophenone for the most sensitive persons was established by N. B. Imasheva at a level of 0.01 mg/m$^3$.

The odor threshold of a phenol-acetophenone mixture was determined on 18 subjects. Of these, 6 persons who were the most sensitive in the tests performed participated in experiments involving both isolated action of the compounds and the mixture of phenol and acetophenone. A total of 752 determinations were made. In studying the action of the mixture, its total concentration was expressed in fractions of the threshold concentrations according to the given test. Studies involving the determination of the odor threshold of the phenol-acetophenone mixture for the most sensitive persons gave concurrent results.

The odor threshold for these persons was defined as 0.022 mg/m$^3$ for phenol and 0.01 mg/m$^3$ for acetophenone.

The odor threshold mixture had a total concentration index of 0.99 $\left(\frac{0.013\ \text{mg/m}^3}{0.022\ \text{mg/m}^3} + \frac{0.004\ \text{mg/m}^3}{0.01\ \text{mg/m}^3}\right)$, i. e., when low concentrations of phenol and acetophenone vapors are present together in atmospheric air, a complete summation of their action takes place.

Expressed in fractions of the thresholds for isolated action, the imperceptible mixture had a total concentration index of 0.72.

The study of the influence of low concentrations of the phenol-acetophenone mixture on the light sensitivity of the eye was made on an ADM adaptometer using a procedure recommended by the Committee on Sanitary Protection of Atmospheric Air. We introduced some modifications of the design of the cylinder and supplied air at a rate of 30-35 l/min. The increased velocity of air movement was not perceived by the subjects. It was thus possible to make the volume of supplied air (500 ml/sec) equal to the volume of air inhaled by the person at rest.

Adaptometric studies were conducted on three subjects. Two mixtures of different concentrations of phenol and acetophenone were tested. A total of 93 determinations were made. Each test lasted 45 minutes, and the light sensitivity of the eyes was measured successively every 5 minutes. Four tests were carried out with each gaseous mixture. The results were subjected to statistical treatment.

In the adaptometric studies, our calculations were made with threshold values obtained by B. M. Mukhitov and N. B. Imasheva for the isolated action of phenol and acetophenone. The total concentration index of the first mixture was equal to 1.0 $\left[\frac{0.00747\ \mathrm{mg/m^3}}{0.0155\ \mathrm{mg/m^3}}\right.$ (phenol) + $\frac{0.00517\ \mathrm{mg/m^3}}{0.01\ \mathrm{mg/m^3}}$ (acetophenone)], and that of the second mixture, to 0.77 $\left(\frac{0.0059\ \mathrm{mg/m^3}}{0.0155\ \mathrm{mg/m^3}} + \frac{0.0039\ \mathrm{mg/m^3}}{0.01\ \mathrm{mg/m^3}}\right)$.

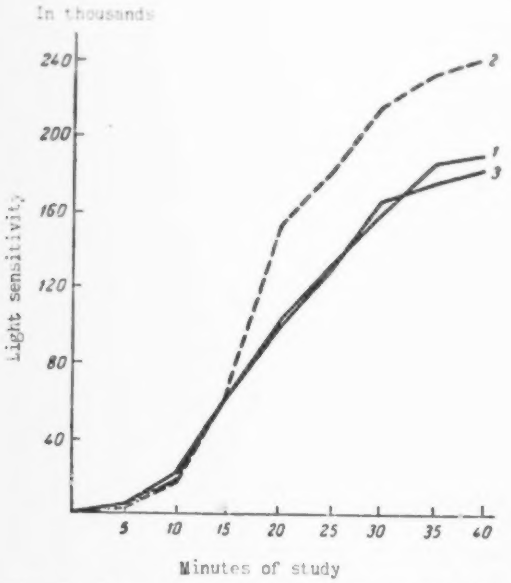


Fig. 1. Curve of dark adaptation of the eyes in subject T. N. during inhalation of gaseous mixture of phenol and acetone.

1 - pure air; 2 - mixture with total concentration index of 1.0; 3 - mixture with total concentration index of 0.77.

In the studies performed, the first mixture caused statistically significant changes in all three subjects. The second mixture caused no changes in the course of the curve of dark adaptation of the eyes (Fig. 1).

Thus, in this experiment as well as in the experiments involving the determination of the threshold of olfactory perception of the phenol-acetophenone mixture, a complete summation of the action of the compounds studied was observed again: the total concentration index of the minimum active mixture was equal to 1.

We studied the influence of low concentrations of the phenol-acetophenone mixture on the electrical activity of the cerebral cortex by using a procedure developed for sanitary investigations by A. D. Semenenko. We used the method of reinforcement of man's intrinsic alpha rhythm during successive stimulation of the subject with intermittent light corresponding to the frequency of his rhythm and to the procedure similarly described in the paper of U. G. Pogosyan. The studies were conducted on three subjects (112 tests).

The leads were taken from each hemisphere from the occiput, temple, and forehead. Two mixtures of gaseous concentrations of phenol and acetophenone were tested. First mixture: phenol - $\frac{0.00759 \text{ mg/m}^3}{0.0156 \text{ mg/m}^3} = 0.49$ (in fractions of the threshold concentration for isolated action) and acetophenone - $\frac{0.00357 \text{ mg/m}^3}{0.007 \text{ mg/m}^3} = 0.51$. The total concentration index of the mixture was equal to 1. Second mixture: phenol - $\frac{0.00584 \text{ mg/m}^3}{0.0156 \text{ mg/m}^3} = 0.374$, and acetophenone - $\frac{0.00276 \text{ mg/m}^3}{0.007 \text{ mg/m}^3} = 0.394$. The total concentration index was 0.77 (Fig. 2). In the electroencephalographic studies performed, the calculations involved the use of the thresholds of individual action of phenol and acetophenone established by B. Mukhitov and N. B. Imasheva. The first mixture caused statistically significant changes in all three subjects, whereas the second mixture did not cause any significant changes. The experiment performed confirms the complete summation of the action of phenol and acetophenone.

When the total concentration of the mixture of phenol and acetophenone was expressed in fractions of their highest single maximum permissible concentrations for isolated action, it was found that the minimum active concentration was 1.96, and the maximum inactive concentration, 1.51. Thus, when the vapors of phenol and acetophenone are present together in atmospheric air, their total concentration in fractions of the maximum permissible ones should not exceed 1.5.

K. A. Bushtuyeva (1960), V. M. Styazhkin (1962), and B. K. Baykov (1964), who studied the combined action of certain atmospheric pollutants, also established a summation effect when these pollutants were simultaneously present in atmospheric air.

In order to study the resorptive action of phenol-acetophenone mixtures, we carried out a prolonged round-the-clock inhalational exposure of the experimental animals. In the course of the experiment, mixtures of the compounds were studied in the following average concentrations:

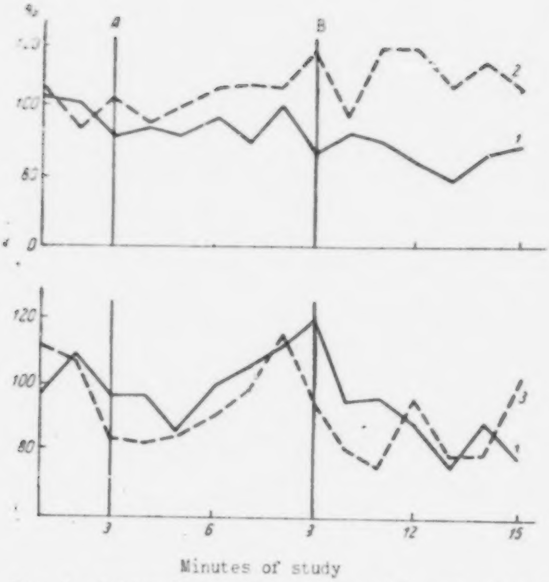


Fig. 2. Change in the electrical activity of the brain in subject T. N. during inhalation of a gaseous mixture of phenol and acetophenone.

Notation same as in Fig. 1; AB - period of inhalation of gaseous mixture.

Group I - acetophenone (0.00171 mg/m$^3$) + phenol (0.0062 mg/m$^3$).

The total concentration index of this mixture, expressed in fractions of the existing mean daily maximum permissible concentrations, was 1.19.

Group II - acetophenone (0.01732 mg/m$^3$) + phenol (0.0637 mg/m$^3$).

The total concentration index of the mixture was 12.14.

Group III - control.

In addition, in groups IV and V, in collaboration with Graduate Student N. Z. Tkach, we studied the effect of three compounds: acetophenone, phenol, and acetone.

Group IV - acetophenone (0.00147 mg/m$^3$) + phenol (0.0048 mg/m$^3$) + acetone (0.136 mg/m$^3$).

The total concentration index of the mixture was 1.36.

Group V - acetophenone (0.01141 mg/m$^3$) + phenol (0.04162 mg/m$^3$) + acetone (1.334 mg/m$^3$).

The total concentration index of the mixture was 11.76.

The studies were conducted on white male rats with an initial weight of 70-100 g. Fifteen animals were placed in each exposure chamber. The concentrations of the substances studied were checked daily during the 84 days of exposure. During that time, observations were made on the general condition of the rats, their weight, the cholinesterase activity, motor chronaxy of antagonist muscles, porphyrin metabolism, number of eosinophils in peripheral blood, and excretion of 17-ketosteroids and vitamin C with the urine.

The general condition of the animals and their weight in all the groups did not differ in any way from those of animals of the control group.

We determined the cholinesterase activity by using a slightly modified procedure of Fleisher, Pope and Spear (1954). A statistically significant decrease in cholinesterase activity was detected on the 4th day of exposure in animals of groups II and V, exposed to high concentrations of the compounds studied. The changes remained distinct until the end of the experiment, and the cholinesterase activity returned to normal only during the recovery period. In the animals of the remaining groups, no such disturbances arose (Fig. 3).

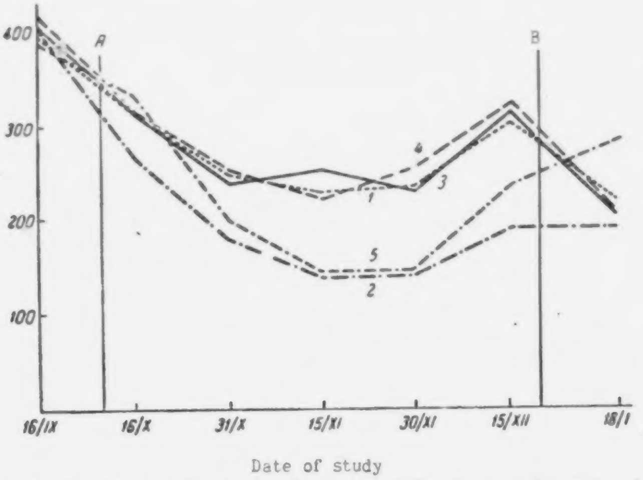


Fig. 3. Changes in cholinesterase activity in experimental animals during inhalational exposure to mixtures of phenol, acetophenone, and acetone.
1 - group I (mixture of phenol and acetophenone with total concentration index of 1.19); 2 - group II (mixture of phenol and acetophenone with total concentration index of 12.14); 3 - group III (control); 4 - group IV (mixture of phenol, acetophenone, and acetone with total concentration index of 1.36); 5 - group V (mixture of phenol, acetophenone, and acetone with total concentration index of 11.76); AB - period of exposure.

We were able to evaluate the functional state of the nervous system from data on the motor chronaxy of antagonist muscles, which was studied in the course of the chronic experiment. As we know, shifts in motor chronaxy, which is a fairly mobile indicator of the excitability and changes under the influence of the slightest factors of the surrounding medium acting on the organism, accurately characterize the functional state of the organism. Exposure of the animals to high concentrations of the substances studied (groups II and V) had a considerable influence on the functional state of the nervous system (Fig. 4). A change in motor chronaxy appeared in these groups toward the end of the first month of exposure. There was no change in the remaining groups of animals. At the end of the recovery period, the ratio of chronaxies returned to normal

Various intoxications and illnesses affecting the nervous system cause disturbances in the porphyrin metabolism. The appearance of coproporphyrin in the urine indicates incipient manifestations of a disturbance of nervous regulation in the synthesis of hemoglobin (O. M. Chernyy and S. E. Krasovitskaya, 1951). In our studies, a significant increase in the excretion of coproporphyrin with the urine was observed in animals of groups II and V during three months of exposure. During the recovery period, the excretion of coproporphyrin became normal (Fig. 5).

In the course of the chronic experiment, we also observed the excretion of 17-ketosteroids with the urine. A leading role in the regulation of excretion of steroid hormones of the adrenal cortex, which possess a marked reactivity, is played by the nerous system, and the function of the cortical part of the adrenals depends on the corticotrophic hormone of the anterior hypophyseal lobe, which in turn is controlled by the central nervous system (S. G. Genes, 1955). The corticosteroid function is a response to a stimulus acting on the organism. The study of 17-ketosteroids gives an idea of the disturbance of the mechanisms controlling the function of the adrenal cortex. The adrenal hormones influence the morphological composition of perpiheral blood. This is particularly apparent in the eosinophils. Eosinopenia serves as an indicator of the reinforcement of the function of the adrenal cortex (D. Ya. Shchurygin, S. F. Murchakova, and N. A. Belov, 1957).

We therefore investigated the excretion of 17-ketosteroids and the number of eosinophils in the blood during exposure of the rats. Changes in the excretion of 17-ketosteroids with the urine were observed in animals of groups II and V. It can be seen from Fig. 6 that an increased excretion of the hormones in these groups corresponds to the entire period of the chronic experiment, but statistically significant changes were obtained only in the third month of exposure, which may be accounted for to some extent by the irregularity of the excretion of 17-ketosteroids by the control group of the animals. The content of 17-ketosteroids in the urine of animals of groups I and IV differed only slightly from that of animals of the control group. The analyses were made every 15 days at a fixed time.

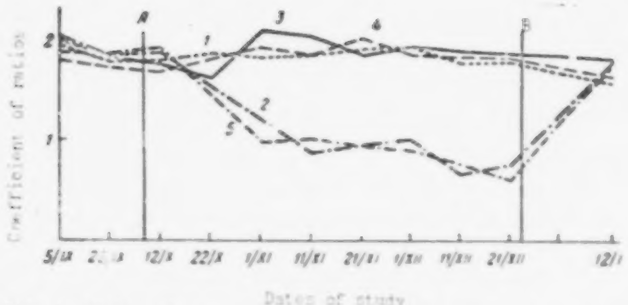


Fig. 4. Ratio of chronaxies of extensors and flexors in experimental animals during inhalational exposure to mixtures of phenol, acetophenone, and acetone.
Notation same as in Fig. 3.

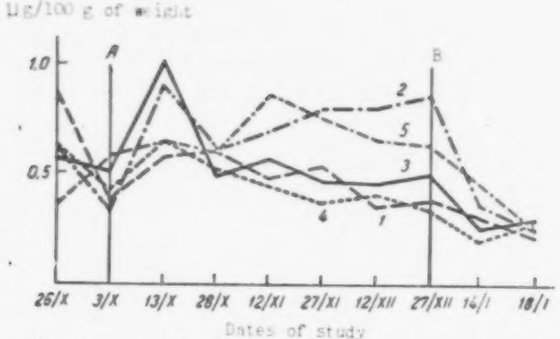


Fig. 5. Excretion of coproporphyrin with the urine during inhalational exposure of experimental animals to mixtures of phenol, acetophenone, and acetone.
Notation same as in Fig. 3.

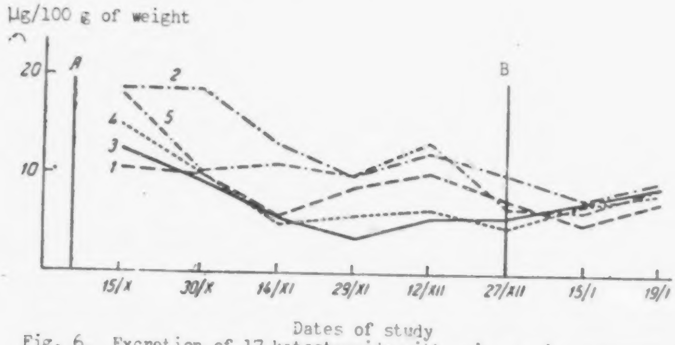
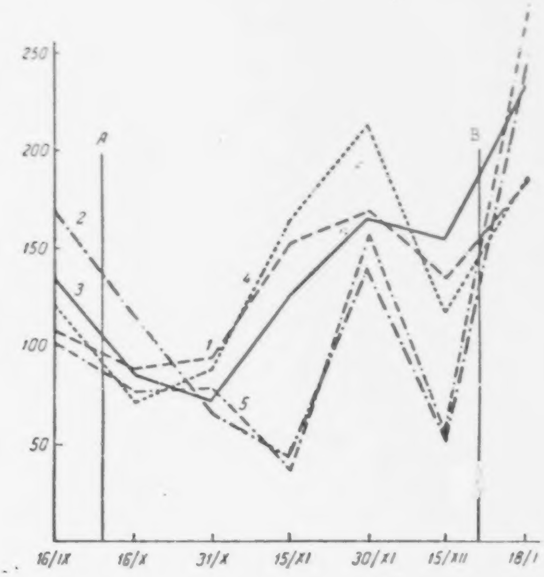


Fig. 6. Excretion of 17-ketosteroids with urine during exposure of experimental animals to mixture of phenol, acetophenone, and acetone.
Notation same as in Fig. 3.

The dynamic study of the content of eosinophils in peripheral blood may serve as one of the indicators of the reactivity of the organism. The most diverse factors leading to the development of inhibition in the central nervous system cause a decrease of the absolute number of eosinophils in peripheral blood (K. Kh. Kyrge, 1956). The eosinophils were counted in a Fuchs-Rosenthal counting chamber (S. M. Bakman, 1958). A decrease in the number of eosinophils in the peripheral blood of animals of groups II and V was observed. Eosinopenia, confirmed statistically, was detected twice in the second and third months of exposure (Fig. 7). No significant changes in the fluctuation of eosinophils were observed in animals of the remaining groups.

The determination of the content of ascorbic acid in the urine of animals of all the groups failed to show any changes as compared with the control.

Thus, inhalational exposure of experimental animals to a mixture of high concentrations of phenol and acetophenone (group II) and their combination with acetone (group V) clearly showed changes in cholinesterase activity, motor chronaxy of antagonist muscles, porphyrin metabolism, excretion of 17-ketosteroids with urine, and a drop in the eosinophil count in the peripheral blood of the animals.



Dates of study

Fig. 7. Fluctuations of eosinophils in the peripheral blood of the animals during inhalational exposure to mixtures of phenol, acetophenone, and acetone.
Notation same as in Fig. 3.

No changes in the organism of the animals during prolonged exposure were caused by total concentrations of phenol and acetophenone at a level of 1.19 in fractions of the existing maximum permissible concentrations and of 1.36 in the combination of these compounds with acetone.

Considering that the gap between our proposed highest single concentration of the phenol-acetophenone mixture (1.5) and the investigated mean daily concentrations (1.19 and 1.36) is small, and that the minimum resorptively acting mixture lies at a level of 1.96, in accordance with the recommendation of the Section on Sanitary Protection of Atmospheric Air of the All-Union Problem Commission, we propose a mean daily concentration of the investigated compounds at the same level as the highest single concentration, i. e., 1.5 in fractions of the maximum permissible concentrations for isolated action.

One of the sections of our work consisted in a study of the actual pollution of atmospheric air around a plant producing synthetic phenol and acetone, and in a sanitary evaluation of the data obtained. The samples were collected in May 1964 on the leeward side of the plant at distances from 100 to 1000 m. According to the data of the municipal sanitary-epidemiological station, the plant discharges into the atmosphere 2.2 tons of pure phenol per year. The discharges of acetophenone are not taken into account. At a distance of 500 m, the highest single concentrations of phenol surpassed the maximum permissible concentrations by a factor of 8.7, and those of acetophenone, by a factor of 9.3 (Table 3). The total concentration of the investigated compounds surpasses our recommended highest single concentration by a factor of 12. At a distance of 1000m, neither of these compounds was observed in atmospheric air. The width of the sanitary protective zone for this type of production can be established only after analyzing the atmospheric air for the entire assortment of the toxic substances discharged by the plant.

Table 3

Pollution of Atmospheric Air Around a Plant Producing Synthetic Phenol and Acetone

| Distance From Source, m | Number of Samples | Of These, Above The Sensitivity of the Method | Concentrations, mg/m$^3$ | |
|---|---|---|---|---|
| | | | Maximum | Average |
| | | Phenol | | |
| 100 | 17 | 17 | 0,29 | 0,1642 |
| 300 | 13 | 13 | 0,219 | 0,1019 |
| 500 | 23 | 20 | 0,087 | 0,03417 |
| 1000 | 25 | — | — | — |
| | | Acetophenone | | |
| 100 | 11 | 11 | 0,879 | 0,258 |
| 300 | 17 | 17 | 0,0967 | 0,0571 |
| 500 | 26 | 12 | 0,0286 | 0,00764 |
| 1000 | 25 | — | — | — |

## Conclusions

1. The odor threshold mixture of phenol and acetophenone for the most sensitive persons is one of 0.013 mg/m$^3$ phenol and 0.004 mg/m$^3$ acetophenone with an index of total concentration in fractions of threshold concentrations for isolated action equal to 1. A mixture of 0.01 mg/m$^3$ phenol and 0.0026 mg/m$^3$ acetophenone with a total concentration index of 0.72 is imperceptible.

2. The threshold mixture from the standpoint of the light sensitivity of the eyes is one consisting of 0.00747 mg/m$^3$ phenol and 0.00517 mg/m$^3$ acetophenone with a total concentration index of 1. The total concentration index of the inactive mixture is 0.77.

3. A mixture of 0.00759 mg/m$^3$ phenol and 0.00357 mg/m$^3$ acetophenone with a total concentration index of 1 is active with respect to the electrical activity of the brain.

A mixture with a total concentration index of 0.77 is inactive.

4. The studies performed show that when phenol and acetophenone are jointly present in atmospheric air, a complete summation of their action takes place.

5. Since the maximum permissible concentrations of phenol and acetophenone were established with a certain margin, their highest single total concentration expressed in fractions of the corresponding maximum permissible concentrations should not exceed 1.5.

6. Exposure of experimental animals to phenol concentrations of 0.0637 mg/m$^3$ and acetophenone concentrations of 0.01732 mg/m$^3$ (total concentration index 12.14) and to a mixture of phenol, acetophenone, and acetone with a total concentration index of 11.765 revealed substantial changes in cholinesterase activity, motor chronaxy of antagonist muscles, porphyrin metabolism, and content of 17-ketosteroids in the urine of the animals, and caused a marked eosinopenia.

7. A mixture of phenol and acetophenone and also their combination with acetone does not cause any changes in the animal organism during a prolonged exposure if their total concentration in fractions of the maximum permissible values does not exceed 1.19 and 1.36 respectively.

8. The mean daily maximum permissible concentration of phenol and acetophenone present simultaneously in atmospheric air may be recommended at the same level as the highest single concentration.

This conclusion also applies to the combined action of the three compounds.

9. The width of the sanitary protective zone for a plant producing synthetic phenol and acetone can be established after a complete analysis of atmospheric air for all of the toxic substances discharged by the plant.

# LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

# SANITARY EVALUATION OF THE COMBINED ACTION OF ACETONE AND PHENOL IN ATMOSPHERIC AIR

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 135-154, (1967).

Experimental studies of the biological effect of atmospheric pollutants have been greatly expanded in the Soviet Union and have formed the basis for the standardization of the maximum permissible content of noxious substances in atmospheric air. A list of such norms currently specifies the maximum permissible concentrations of 67 ingredients for isolated action.

However, the discharges of a single enterprise frequently contain several noxious substances, and the surrounding atmosphere is polluted by several of them simultaneously. The substances used in industry are very complex mixtures of various compounds; the different ingredients may react with each other and form new, sometimes more toxic compounds. For example, a single populated area frequently contains several different kinds of industrial plants discharging different substances, etc. For this reason, in addition to developing further the studies of various ingredients on the organism, it is necessary to investigate the action of their mixtures.

From the standpoint of their combined action, atmospheric pollutants have been studied comparatively little. Research along these lines has shown that when certain substances are simultaneously present in air, a complete summation of their action is observed: such are sulfur dioxide and sulfuric acid aerosol (K. A. Bushtuyeva, 1961); hydrogen sulfide, carbon disulfide and Dowtherm (Kh. Kh. Mannanova, 1964); ethylene, propylene, butylene and amylene (M. L. Krasovitskaya, 1964); isopropylbenzene and its hydroperoxide (G. I. Solomin, 1964); strong mineral acids (sulfuric, hydrochloric, and nitric) in concentrations of H ions (V. P. Melekhina, 1964). The results of these studies are embodied in a table of maximum permissible concentrations of noxious substances in the atmospheric air of populated areas, ratified at the end of 1965.

One of the most promising technological processes that have emerged in the world industry of organic synthesis is the method of combined production of acetone and phenol via isopropylbenzene hydroperoxide (cumene). It was first developed and applied on an industrial scale in the Soviet Union in 1949. Because of the steadily increasing consumption of acetone and phenol in various branches of the national economy, the cumene method of production of these compounds, which is the most economical method, is being widely adopted. Enterprises engaged in the combined production of acetone and phenol by the cumene process are among those which can cause pollution of the atmosphere with noxious substances and their combinations because of imperfections from the standpoint of sanitary protection of the outside environment, imperfections in the technological processes, and an inadequate development of methods of purification of the discharges.

Studies have shown (V. P. Melekhina and M. A. Pinigin, 1961) that the atmospheric air around a plant of combined acetone and phenol production is polluted with acetone, phenol, and many other substances at a considerable distance from the plant. The study of the combined action of acetone and phenol in low concentrations is of definite practical and theoretical interest because of the necessity of standardizing the content of these compounds in atmospheric air when they are jointly present. Our study correlates data obtained in an experimental study of this problem.

Acetone (dimethyl ketone) is an irritating and narcotic compound which has the property of accumulating in the organism. Phenol (carbolic acid) is a nerve poison which has a local irritating and cauterizing effect (N. V. Lazarev, 1951). In determining acetone and phenol in atmospheric air, we used a nephelometric method for acetone (M. V. Alekseyeva, B. Ye. Andronov, S. S. Gurvits, and A. S. Zhitkov, 1954) and a colorimetric method for phenol (V. A. Khrustaleva, 1962).

Acetone reacting with iodine in an alkaline medium forms a white suspension of iodoform, whose intensity is compared with a standard scale. The sensitivity of the method is 0.001 mg in a volume of 4.5 ml. The method is specific, and phenol does not interfere with the determination.

When phenol reacts with 4-aminoantipyrine in the presence of potassium ferricyanide at pH 9.3, a pink color appears. The sensitivity of the method is 0.0002 mg in a volume of 2 ml. Acetone does not interfere with the determination.

We began our study of the effect of low concentrations of acetone and phenol on the human organism by determining the threshold of their olfactory perception in a mixture, having first verified the already-established odor thresholds of the separate substances in 18 persons.

The determination of the threshold of olfactory perception was performed by using a standard procedure (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. V. Novikov, 1957). Of 18 people, 5 were selected for whom the odor thresholds of both acetone and phenol were the lowest and amounted to 1.1 mg/m$^3$ for the former compound and 0.022 mg/m$^3$ for the latter; this confirms some studies made earlier (Yu. G. Fel'dman, 1962; B. A. Mukhitov, 1963). The concentration of acetone and phenol in the determination of the threshold of olfactory perception of their mixture is given in Table 1.

Table 1

Concentration of Acetone and Phenol Corresponding to the Threshold of Olfactory Perception of Their Mixture.

| Concentration of Acetone and Phenol in Their Mixture | | | | Total Concentration in Fractions of the Threshold Concentration of each Component Part |
|---|---|---|---|---|
| mg/m$^3$ | | In Fractions of Threshold for Isolated Action | | |
| Acetone | Phenol | Acetone | Phenol | |
| $\frac{1{,}1^{*}}{1{,}1}$ | $\frac{0{,}022}{0{,}022}$ | 1,0 | 1,0 | 2,0 |
| $\frac{0{,}72}{1{,}1}$ | $\frac{0{,}011}{0{,}022}$ | 0,65 | 0,5 | 1,15 |
| $\frac{0{,}55}{1{,}1}$ | $\frac{0{,}011}{0{,}022}$ | 0,5 | 0,5 | 1,0 |
| $\frac{0{,}55}{1{,}1}$ | $\frac{0{,}008}{0{,}022}$ | 0,5 | 0,36 | 0,86 |
| $\frac{0{,}36}{1{,}1}$ | $\frac{0{,}011}{0{,}022}$ | 0,33 | 0,5 | 0,83 |

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* Numerator - concentration studied; denominator - odor threshold concentration for isolated action.

We shall illustrate the nature of the combined action of the substances studied by using as an example the data obtained on the five most sensitive persons (Table 2).

As can be seen from Table 2, the odor threshold of the acetone-phenol mixture for the five most sensitive persons was established for an index of 1.0 of the total concentration in fractions of the threshold concentration for each component part. The remaining 13 indicated concentrations were not perceived by the subjects, which is quite natural, since they had a higher odor threshold for both compounds taken both separately and in a mixture.

The perception threshold is not the limit of the physiological activity of acting stimuli. Very frequently, without being accompanied by sensations, stimulations cause definite physiological responses of so-called subliminal character (G. V. Gershuni, 1949). This phenomenon was also confirmed in studies with low concentrations of noxious substances in the inhaled air. Studies made along these lines showed that subliminal concentrations of chemical stimuli whose odor is imperceptible cause changes in the functional state of different organs and systems, particularly the visual system, and the threshold of these changes is slightly below the threshold established by the interrogation method.

Table 2

Thresholds of Olfactory Perception of an Acetone-Phenol Mixture for the Most Sensitive Persons.

| Total Concentration in Fractions of Threshold Concentration of Each Component Part | Results of Tests | |
|---|---|---|
| | Perceive | Do Not Perceive |
| 2,0 | 5 Persons | — |
| 1,15 | 5 » | — |
| 1,0 | 5 » | — |
| 0,86 | — | 5 Persons |
| 0,83 | — | 5 » |

The light sensitivity is an extremely delicate and labile function of the visual system, whose level is affected by various factors in the surroundings. There is special merit to the hypothesis, based on a series of studies (L. A. Orbeli, 1934; A. V. Lebedinskiy, 1935; K. Kh. Kekcheyev, 1946; P. P. Lazarev, 1947; S. V. Kravkov, 1950), that the light sensitivity of the eyes at the level of total dark adaptation reflects not only the processes taking place in the receptor itself, but is intimately tied up with the state of the visual centers of the cerebral cortex and its stem part. Hence, a change in the level of the light sensitivity of the eye under the influence of secondary stimuli should be regarded as being a reflection of physiological shifts of not only peripheral but also central origin.

A study of the reflex effect of an acetone-phenol mixture on the light sensitivity of the eyes under dark adaptation conditions was made by using an ADM adaptometer according to a standard procedure on 3 out of 5 persons with the most sensitive odor threshold. All three had a normal vision and a comparatively stable dark adaptation curve. In view of the fact that for the most sensitive persons the odor threshold concentrations in our studies coincided with the concentrations established earlier (Yu. G. Fel'dman, 1962; B. M. Mukhitov, 1[illegible]), in carrying out the adaptometric tests we used the thresholds of the reflex effect of acetone and phenol on the light sensitivity of the eyes already established by these authors.

The first combination with a total concentration index of 1.6 causes statistically significant changes of the light sensitivity of the eyes in all three subjects. The second combination with a total concentration index of 1 caused statistically significant changes of the light sensitivity of the eyes in only two persons. It proved inactive for the third subject.

An acetone-phenol mixture with a total concentration index of 0.85 was found to be inactive.

Table 3

Ratio of Acetone and Phenol Concentrations in the Determination of the Threshold of Their Reflex Effect on the Light Sensitivity of the Eye

| Concentration of Acetone and Phenol in the Mixture | | | | |
|---|---|---|---|---|
| In mg/m$^3$ | | In Fractions of Threshold for Isolated Action | | Total Concentration in Fractions of Threshold Concentration of each Component Part |
| Acetone | Phenol | Acetone | Phenol | |
| $\frac{0{,}44^*}{0{,}55}$ | $\frac{0{,}0125}{0{,}0156}$ | 0,8 | 0,8 | 1,6 |
| $\frac{0{,}27}{0{,}55}$ | $\frac{0{,}0078}{0{,}0156}$ | 0,5 | 0,5 | 1,0 |
| $\frac{0{,}22}{0{,}55}$ | $\frac{0{,}0070}{0{,}0156}$ | 0,4 | 0,45 | 0,85 |

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* Numerator concentrations studied; denominator - threshold concentration for the given test for isolated action.

Thus, the threshold of the reflex effect of acetone and phenol on the light sensitivity of the eye is at the level of a total concentration index equal to 1. A mixture containing acetone and phenol in concentrations amounting to less than 1 does not act on the light sensitivity of the eye.

The response of the light sensitivity of the eyes in our subjects to a brief inhalation of acetone and phenol in a mixture was marked by individual characteristies. Thus, in subjects R. L. and Ye. S., the changes were manifested in a decrease of the light sensitivity of the eyes, and in subject G. L. the light sensitivity of the eyes increased.

In studies involving the experimental standardization of atmospheric pollutants, the method of electroencephalography has come into wide and justified use. Being a highly sensitive indicator of the functional state of the nervous system, the electroencephalographic method best satisfies the requirements arising in the solution of theoretical problems and in practical hygienic standardization of noxious substances in atmospheric air.

We conducted a study of the reflex change of the electrical activity of the brain during inhalation of a mixture of acetone and phenol on three subjects by using a quantitative analysis of the reflex response of reinforcement of the intrinsic alpha rhythm (A. D. Semenenko, B. N. Balashov, and Ye. V. Arzamastsev, 1963).

The thresholds of the reflex effect of acetone and phenol on the energy of the brain potentials were established at the level of 0.44 mg/m$^3$ (Yu. G.

Fel'dman, 1962) and 0.0155 mg/m$^3$ (B. M. Mukhitov, 1963) respectively. Our tests utilized these threshold values, established on the basis of desynchronization of the alpha rhythm during the development of the electrocortical conditioned reflex. The tests were based on the use of the dependence of the nature of the electroencephalogram on the functional state of the brain. Principal attention was focused on the response of a burst of the alpha rhythm and on the changes arising against the background of this burst as a result of inhalation of an aceton-phenol mixture.

The stimulus used was light flickering rhythmically at a frequency equal to the optimum frequency of the subject's alpha rhythm.

All the subjects underwent training experiments for 4 weeks in order to make them develop a well-defined alpha rhythm and a definite dynamic stereotype. The main observations were begun after obtaining comparatively stable data during inhalation of pure air. This was accomplished by using standard, stable conditions of observations. During the tests, the subject had electrodes attached to the surface of his head in a faintly illuminated, soundproof chamber, rested in a comfortable semireclining position, and breathed pure air supplied at a rate of 30 l/min from a cylinder. Two pickup units recording the motor response to the stimuli were attached to the wrists. All the subjects had been given preliminary instructions. The electroencephalogram was recorded in the following sequence: first, the initial background was recorded while pure air was being supplied. Starting with the 4th minute, the subject breathed the mixture studied for 6 minutes, then pure air was again supplied for the next 6 minutes (recovery period). The entire observation lasted 18 minutes, including 3 minutes of training. During each minute, the rhythmic light was given for 18 seconds, and its intensity was changed every 5 seconds. In the intervals separating the times when the light was turned on, the subject made free movements in his chair (limbered up). A sound whose intensity was also varied was then turned on for 10 seconds. After the sound was discontinued, the subject waited for the light for 7 seconds.

During the entire study, the subject was in a state of active mental and motor activity. This removed the possibility of the onset of phase states. It is known that functional loads make it possible to identify very delicate shifts arising in the tissues and organs. Indeed, the functional load we used, the photic and sonic stimuli, their differentiated analysis, and the response to these stimuli formed the background for the identification of the shifts caused by the reflex effect of low concentrations of acetone and phenol whose odor was imperceptible and which did not act on the light sensitivity of the eye. These changes occurred against a background of the reinforced intrinsic alpha rhythm of the subjects.

In our studies, the electric potentials were recorded bipolarly on a 16-channel Galileo electroencephalograph from the frontal, temporal, and parietal regions and their combinations from both cerebral hemispheres. The subject's response to changes in the intensity of the photic and sonic stimuli (pressing on the piezoelectric pickup) was also recorded. The electroencephalogram of each observation was analyzed on the basis of the amplitude of the reinforced intrinsic alpha rhythm expressed in microvolts, i. e., from the integrated energy of the brain potentials. To perform a comparative analysis of the results obtained, the total amplitude of the reinforced intrinsic alpha rhythm was expressed in percent. The average total amplitude for the first 3 minutes taken as the background was taken as 100%, and the total amplitudes in the remaining minutes were compared with it (A. D. Semenenko, 1963). The results of these studies were treated statistically, i. e., the degree of significance of the shifts obtained were found. The ratio of the investigated and threshold concentrations is given in Table 4.

Table 4

Ratio of Concentrations of Acetone and Phenol in the Determination of the Threshold of Their Reflex Effect on the Electrical Activity of the Brain

| Concentration of Acetone and Phenol in the Mixture | | | | |
|---|---|---|---|---|
| In mg/m$^3$ | | In Fractions of the Threshold for Isolated Action | | Total Concentration in Fractions of the Threshold Concentration of Each Component Part |
| Acetone | Phenol | Acetone | Phenol | |
| $\frac{0{,}22^*}{0{,}44}$ | $\frac{0{,}0078}{0{,}0156}$ | 0,5 | 0,5 | 1,0 |
| $\frac{0{,}17}{0{,}44}$ | $\frac{0{,}0068}{0{,}0156}$ | 0,39 | 0,44 | 0,83 |

* Numerator-Concentration studied; denominator - threshold concentration for the given test for isolated action.

A mixture with a total concentration index equal to 1 acts on the energy of the brain potentials, causing statistically significant changes of the reinforced intrinsic alpha rhythm in all the subjects (Fig. 1).

A concentration of acetone and phenol with a total index of less than 1 has no effect on the electrical activity of the brain.

The data obtained from all three tests permit one to draw the conclusion that low concentrations of acetone and phenol present together in the inhaled air produce a complete summation of their action.

When the threshold concentrations obtained by the electroencephalographic method are expressed in fractions of the existing maximum permissible concentrations of the separate ingredients, the minimum effective total concentration is 1.41, and the maximum ineffective concentration, 1.16. Hence, in the joint presence of acetone and phenol in atmospheric air, the sum of their concentrations expressed in fractions of the maximum permissible ones should not exceed 1.

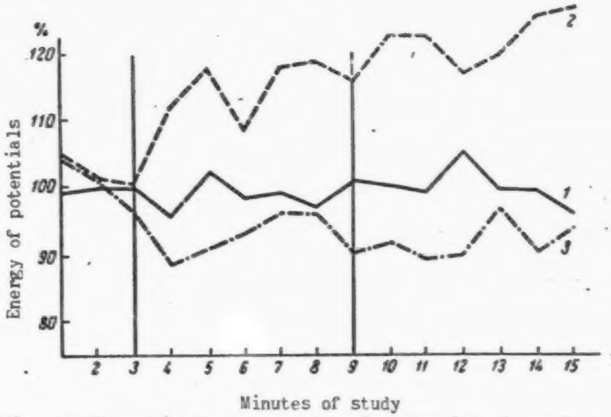

Fig. 1. Change in the energy of brain potentials in subject R. L. under the influence of inhalation of an acetone-phenol mixture.

1 - pure air; 2 - 0.22 mg/m$^3$ acetone and 0.0078 mg/m$^3$ phenol; 3 - 0.17 mg/m$^3$ acetone and 0.0068 mg/m$^3$ phenol.

To detect the resorptive effect of low concentrations of acetone and phenol in a mixture for the purpose of validating their mean daily maximum permissible concentration in atmospheric air, we carried out a chronic three-month exposure of white rats. The 48 males selected for the experiment, with an initial weight ranging from 90 to 110 g, were divided into three groups of 16 animals each.

Group I of the animals was exposed to a mixture of acetone and phenol in concentrations 10 times greater than the maximum inactive mixture for brief inhalation. On the average, it amounted to 1.657 ± 0.0215 mg/m$^3$ - acetone and 0.0545 ± 0.0013 mg/m$^3$ - phenol; the total concentration index expressed in fractions of the maximum permissible concentrations of each ingredient for isolated action was equal to 10.19.

Group II of the animals was exposed to a mixture in concentrations at the level of the maximum inactive mixture for brief inhalation. On the average, it amounted to 0.1735 ± 0.00305 mg/m$^3$ - acetone and 0.00516 ± 0.0000769 mg/m$^3$ - phenol; the total concentration index in the same fractions was equal to 1.012.

Group III of the animals served as the control.

During the entire course of exposure and of the recovery period, the animals were subjected to the same conditions. Pure air and air containing acetone and phenol were supplied to the exposure chambers at a rate of 35 l/min. The air from the chambers was checked daily for its content of acetone and phenol. To estimate the toxic effect, the general state and behavior of the animals was followed over the course of the entire experiment. During the entire period of exposure, this observation failed to detect any shifts in these indices in animals of the experimental groups as compared with the control. No symptoms indicating a specific effect of acetone and phenol were observed.

The animals were weighed every 20 days. Observation of the weight dynamics showed that animals of all the groups gained weight in approximately the same manner, but toward the end of the exposure and of the recovery period a slight weight lag in animals of the control group was noted.

During the chronic experiment, we studied the motor chronaxy of antagonist muscles and the activity of blood cholinesterase, and determined the content of coproporphyrin and certain vitamins - C, $B_1$, $B_2$ - and N-methylnicotinamide in the daily urine.

In selecting the indicated tests for the study of the character of the resorptive effect of low acetone and phenol concentrations on the organism of the animals, we were guided by the assumption that the resorptive effect of low concentrations of noxious substances consists of general, nonspecific shifts that are sometimes of the same type and insignificant. As a rule, these shifts were functional in character and should be regarded as defensive-adaptive responses (V. A. Ryazanov, 1964). The participation of central nervous mechanisms in the formation of these shifts should be considered general and obligatory.

One of the manifestations of the relationship between the nerve centers and peripheral formations is subordination. It consists in the regulation of the functional state of the periphery by higher centers. The removal of the subordinating influence of the central nervous system immediately affects the level of many functional characteristics of the neuromuscular apparatus, particularly its excitability, one of the indices of which is the chronaxy, i. e., the speed with which a physiological response to a stimulus takes place. In this case, a substantial difference is found in antagonist muscles. It is known that the centers of flexors have a higher excitability than those of extensors. In chronaximetry, this is manifested in the fact that the former have a lower chronaxy than the latter. In a normal relationship of the processes of stimulation and inhibition in the cortex of the cerebral hemispheres, a normal ratio of chronaxy of antagonist muscles is observed. It is usually larger than 1. Under the influence of various environmental factors, this ratio changes so that the chronaxies become similar or the ratio is inverted, and its coefficient becomes smaller than 1. This is a manifestation of a weakening of the subordinating influence of the central nervous system, caused by the development of a process of inhibition in the cerebral cortex.

We studied the motor chronaxy of antagonist muscles in 5 rats of each group once every 10 days. A graphic illustration of the results obtained is given in Fig. 2, which shows that a normal ratio of the chronaxies of extensors and flexors was observed in rats of group II and III. In contrast, in animals of group I on the 45th day of exposure, there was a disturbance in the normal ratio of chronaxies, i. e., the chronaxy of the flexors became greater than that of the extensors. These changes were statistically significant and lasted until the end of the exposure. At the end of the recovery period, the ratio of the chronaxy of extensors and flexors in animals of group I returned to normal.

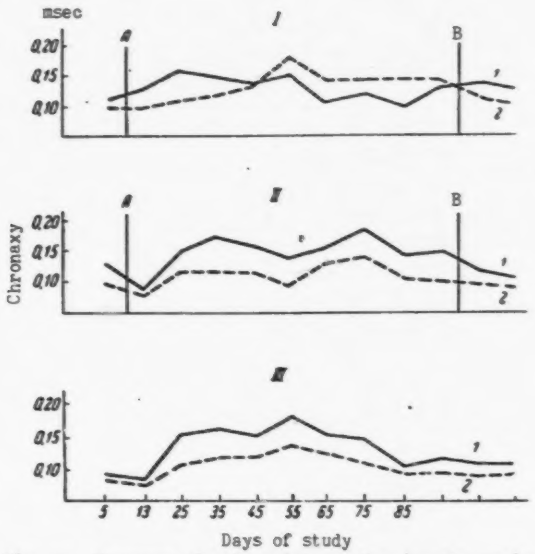

Fig. 2. Chronaxy of antagonist muscles in rats during exposure to a mixture of acetone and phenol (group averages).
I, II and III - groups of animals; 1 - extensors; 2 - flexors; AB - period of exposure.

The creation of a biological equilibrium between the requirements of the organism and the activity of the enzyme systems constitutes one of the mechanisms by which the organism adapts to the living conditions. The biological and chemical transformations in the organism take place with the participation of enzyme systems. One of the enzymes is cholinesterase, which hydrolyzes acetylcholine. Changes in the activity of cholinesterase and in the cholinergic reactions of the blood are one of the indicators of the functional state of nervous activity (D. Ye. Al'pern, 1958). Experimental studies made by D. L. Pevzner (1954) showed that in normal relationships of the basic nervous processes - stimulation and inhibition - the magnitude of cholinesterase activity in the blood serum does not undergo any significant fluctuations. The prevalence of the inhibitory process in the cerebral cortex corresponds to a distinct decrease in cholinesterase activity.

The study of blood cholinesterase activity was carried out in 5 rats of each group every 15 days using the procedure of J. Fleisher and E. Pope, modified by N. N. Pushkina and N. V. Klimkina (N. N. Pushkina, 1963). In rats of group I on the 37th day of exposure, a statistically significant decrease in cholinesterase activity (95% of the blood was observed as compared with the control). This was not observed in animals of group II (Fig. 3).

The literature offers no data on the relative influence of low concentrations of acetone on the activity of blood cholinesterase. According to the data of B. A. Mukhitov (1963), the use of low phenol concentrations causes an increase in the activity of blood cholinesterase. Our studies showed a decrease in cholinesterase activity. This is possibly a manifestation of the specific effect of acetone as a narcotic. It is known from the work of M. Ya. Mikhel'son (1948) that narcotics decrease the activity of cholinesterase. However, given the fact that low concentrations of the substances do not have a specific effect, it is more correct to attribute the results obtained to the nature of the combined action of acetone and phenol and to treat this as a consequence of the formation of an inhibitory center in the cerebral cortex.

A substantial decrease in blood cholinesterase activity was observed only briefly in our studies. Evidently, the compensatory mechanisms rapidly established an equilibrium between the enzyme system and the requirements of the organism produced by the action of the chemical stimulus, the acetone-phenol mixture.

The mechanisms of transformation of energy in the organism of animals and man are based on processes of biological oxidation, i. e., processes of cellular respiration. Biological oxidation is an enzymatic process. The enzymes participating in it constitute an intricate complex of proteins and metalloporphyrins. They determine the respiratory function of the blood. Thus, porphyrins as biologically active substances enter into the composition of hemoglobin, a pigment which is indispensable to the vital functions. In the human organism, there occur a number of transformations of porphyrins, including coproporphyrin with its two isomers, I and III.

The detection of porphyrins in the white matter of the brain and spinal cord is of major interest for gaining an understanding of the nature of the physiological processes occurring in the nervous system. According to a plausible hypothesis, in areas of the central nervous system where the cytochrome is either absent or present in insignificant amounts (white matter of the brain), the oxidation processes are carried out with the aid of porphyrins; and the fact that porphyrins are good hydrogen acceptors and donors should by considered experimentally proven.

Clinical observations and experimental studies by Yu. K. Smirnov (1953) lead to the assumption that porphyrin metabolism is one of the indicators reflecting the functional state of the central nervous system: an intensification of this metabolism takes place when the hypothalamic region of the brain is stimulated. Inhibitory processes in the brain decrease its functional activity and cause a decrease of porphyrin metabolism. All this confirms the hypothesis that the determination of the content of porphyrin and its transformations in the daily urine may serve as an objective test for evaluating the functional state of the central nervous system. This explains the wide use of this test in recent years in experimental studies aimed at validating the maximum permissible content of noxious substances in atmospheric air.

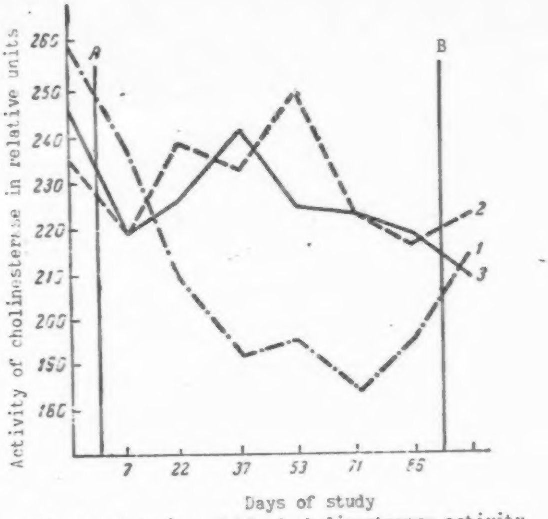

Fig. 3. Dynamics of blood cholinesterase activity in rats during exposure to an acetone-phenol mixture (group averages).
1 - group I; 2 - group II; 3 - group III; AB - period of exposure.

We determined the coproporphyrin content in the combined urine of 5 rats of each group once every 12 days, using the procedure of M. I. Gusev and Yu. K. Smirnov (1960). The determinations showed that under the influence of acetone and phenol, the porphyrin metabolism of animals of group I is depressed. In our studies, statistically significant shifts in the porphyrin metabolism occurred during the 8th week of exposure. At the end of the recovery period, the coproporphyrin content of the animals in group I continued to remain low as compared with groups II and III (Fig. 4). Changes in the porphyrin metabolism of animals of group I should be explained in terms of a disturbance of the normal relationship of the basic nervous processes as being a "physiological gauge" of the defense of the animal organism.

We followed the excretion of vitamins C, $B_1$, and $B_2$, and N-methylnicotinamide with the urine. As we know, vitamins are catalysts for the biochemical reactions taking place in the live cell. They participate in metabolism primarily in the composition of the enzyme system. Thus the determination of the content of vitamins may furnish additional information on the character of the metabolic-enzymatic processes in the organism of experimental animals. The existing literature data point to the observed disturbances of vitamin metabolism in workers employed in the production of phenol and acetone by the cumene process (N. N. Pushkina, 1964), who are exposed to their high concentrations in the air of plant shops.

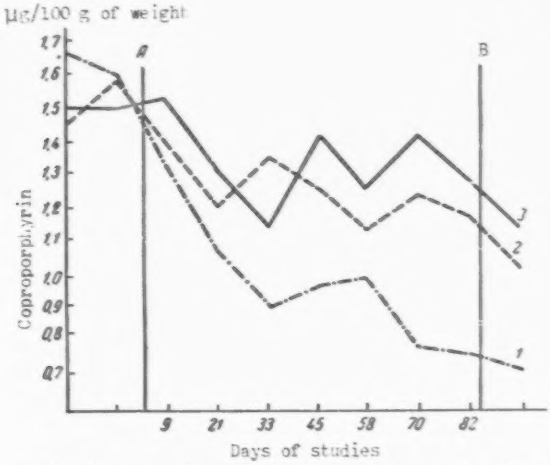

Fig. 4. Dynamics of excretion of coproporphyrin with the urine in rats exposed to an acetone-phenol mixture. Notation same as in Fig. 3

The content of these vitamins was determined in the combined daily urine of 5 rats of each group every 12 days. Shifts in the excretion of the vitamins in animals of the experimental groups were slight and statistically insignificant, and on the 80th day of exposure the content of vitamins in the urine of animals of all the groups was approximately at the same level.

In histopathological studies of animals of group I, the following characteristics were observed: circulatory disturbances in certain parenchymatous organs, edema in the stroma of villi of the small intestine, and change in the permeability of the capillaries with development of moderate perivascular and pericellular edema in the brain.

Thus, the chronic experiment established that in group I of the animals, acetone and phenol in concentrations 10 times greater than the highest inactive mixture for brief inhalation (1.657 mg/m$^3$ acetone and 0.0545 mg/m$^3$ phenol) cause shifts indicating a response of the nervous system: disturbance of the normal ratio of the chronaxy of antagonist muscles, decrease in blood cholinesterase activity, decrease in the content of coproporphyrin in the urine, and also a number of slight anatomico-pathologic changes.

In group II, acetone and phenol in concentrations corresponding to the highest inactive mixture in brief inhalation (0.1735 mg/m$^3$ acetone and 0.00516 mg/m$^3$ phenol) caused no changes in the organism of the animals under the conditions of the chronic experiment.

A study of the resorptive action of the mixture of acetone and phenol also indicates a complete summation of their effects. Hence, the mean daily maximum permissible concentration of acetone and phenol present together may be proposed at the same level as the highest single concentration, i. e., the sum of their fractions of the existing mean daily maximum permissible concentrations for isolated action should not exceed 1.0.

We studied the atmospheric air around a synthetic phenol and acetone plant during the spring of 1964. The air samples to be analyzed for acetone and phenol content were collected on the leeward side at distances of 100, 300, 500 and 1000 m from the plant at a level of 1-1.5 m above ground. Single concentrations of acetone and phenol were determined simultaneously. The results are presented in Table 5 and 6.

Table 5

Single Concentrations of Acetone in Atmospheric Air Around a Plant Producing Synthetic Phenol and Acetone.

| Distance From Source of Discharge, m | Number of Samples | Number of Samples Below the Sensitivity of the Method | Concentration, mg/m$^3$ | |
|---|---|---|---|---|
| | | | Maximum | Average |
| 100 | 14 | — | 2,19 | 1,41 |
| 300 | 13 | — | 0,714 | 0,415 |
| 500 | 24 | 24 | — | — |

Table 6

Single Concentrations of Phenol in Atmospheric Air Around a Plant Producing Synthetic Phenol and Acetone.

| Distance From Source of Discharge, m | Number of Samples | Number of Samples Below the Sensitivity of the Method | Concentration, mg/m$^3$ | |
|---|---|---|---|---|
| | | | Maximum | Average |
| 100 | 17 | — | 0,323 | 0,187 |
| 300 | 13 | — | 0,2197 | 0,115 |
| 500 | 23 | 3 | 0,0869 | 0,039 |
| 1 000 | 25 | 25 | — | — |

As is evident from Table 5 and 6, at a distance of 100 m, the highest acetone concentration surpassed its maximum permissible concentration (0.35 mg/m$^3$) by a factor of 6, and at a distance of 300 m, by a factor of 2. Only at a distance of 500 m was no acetone detected.

At a distance of 100 m from the source of discharge, the highest single phenol concentration was 32 times as high as its maximum permissible concentration (0.01 mg/m$^3$); at 300 m it was 22 times as high, at 500 m, 8 times, and only at a distance of 1000 m was the phenol concentration undetectable. Of practical interest is a comparison of the data obtained with the maximum permissible concentrations of acetone and phenol present together; expressed in fractions of the highest single maximum permissible concentrations for isolated action, they should not exceed 1 in this case. The data presented in Tables 5 and 6 show that a distance of 100 m, the total concentration of acetone and phenol exceeds the maximum permissible value for their combined presence by a factor of 38. At a distance of 300 m, this excess is 24-fold.

Thus, a study of the pollution of atmospheric air around a plant discharging phenol and acetone simultaneously revealed a considerable pollution of the ambient air, particularly with phenol, so that prophylactic measures should be directed primarily at the removal of phenol vapors from the waste gases.

A sanitary-protective zone for plants producing synthetic phenol and acetone can be recommended only by taking into account the pollution of atmospheric air by other noxious substances discharged along with acetone and phenol.

## Conclusion

1. When a mixture of acetone and phenol acts on the sense of smell, light sensitivity of the eye and electrical activity of the human brain, a complete summation of the action of these compounds on the organism is observed.

2. A biological effect of a mixture of acetone and phenol is observed only when their total concentration expressed in fractions of the threshold concentrations for isolated action amounts to less than 1.

3. When acetone and phenol are jointly present in atmospheric air, their highest single concentration should be at the biologically inactive level, i. e., should not exceed 1 in fractions of the highest single maximum permissible concentrations for isolated action.

4. The mean daily maximum permissible concentration of an acetone-phenol mixture in atmospheric air is recommended at the level of the highest single concentration.

# LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of the volume in the 1968 bibliography.

# ON THE COMBINED EFFECT OF LOW CONCENTRATIONS OF ACETONE AND ACETOPHENONE IN AIR ON THE ORGANISM OF MAN AND ANIMALS

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 170-186, (1967).

The rapid development of chemical industry is associated with the discharge of large amounts of toxic substances into the atmosphere. The discharges of a single industrial enterprise most often contain a whole series of noxious substances.

One of the most frequent combinations of atmospheric pollutants are acetone and acetophenone.

The main sources of pollution of atmospheric air by this combination are enterprises that produce synthetic phenol and acetophenone simultaneously by the cumene process. In this method, acetophenone is obtained as a product of side reactions and is also always discharged into the atmosphere.

The combined action of atmospheric pollutants was first studied by K. A. Bushtuyeva (1960) (sulfur dioxide and sulfuric acid aerosol), V. M. Styazhkin (1962) (chlorine and hydrogen chloride), and B. K. Baykov (1963) (carbon disulfide and hydrogen sulfide). In these studies, the authors observed a complete or partial summation of the action of the compounds studied.

The object of our work was to study the effect of low concentrations of acetone and acetophenone on the organism of man and experimental animals when these compounds are jointly present.

Acetone and acetophenone are toxic substances affecting primarily the central nervous system. The toxicology of acetone has been treated in a large number of studies (A. Ye. Yefremov, 1929; N. F. Okuneva, 1930; I. D. Mishenin, 1933; I. S. Tsitovich, 1935; P. M. Sukhov, 1935; N. V. Lazarev, 1954; Yu. G. Fel'dman, 1960; Ch. Khadnan' et al., 1962, and others).

Literature data on the toxicology of acetophenone are very scant (Laborde, 1885; S. S. Kamenskiy, 1889; Friedman and Maase, 1910; N. B. Imasheva, 1963, and others). These investigations deal primarily with the study of the effect of toxic concentrations of acetone and acetophenone. Only the work of Yu. G. Fel'dman on acetone and that of N. B. Imasheva on acetophenone are devoted to the study of the influence of low concentrations in both brief contact and under conditions of a chronic experiment.

In the literature, we were unable to find any data on the combined action of these ingredients.

M. I. Ol'shanskaya and V. V. Likhacheva (1935) studied the combined action of acetone and benzene vapors on pigeons in concentrations of 25,000 and 10,000 mg/m$^3$ respectively. The authors found a complete summation of the action of these compounds.

The mixture with the strongest effect proved to be one containing equal amounts of benzene and acetone.

T. A. Shtessel' (1937) also obtained a simple summation of the effect in a 2- and 4-hour experiment on white mice with combinations of acetone and methanol, acetone and ethyl ether, acetone and toluene, and acetone and benzene. The criterion of the action of each compound taken individually and in combination was the lateral position of the white mice.

A reinforcement of the toxic effect of acetone in combination with benzene is indicated by Nischiama and Isami (1957). The authors found that during the combined action of benzene and acetone, the leucocyte content of the blood decreases even more than during the action of benzene or acetone alone.

We began our experimental studies with the determination of the threshold of olfactory perception of acetone and acetophenone taken separately, then in combination.

The acetone was determined by the nephelometric method, whose sensitivity is 1 µg in a volume of 4.5 ml. Acetophenone was determined spectrophotometrically, with a sensitivity of 0.25 µg in 1 ml.

The odor threshold of acetone was determined in 22 practically healthy people 18 to 48 years old using a standard procedure (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. V. Novikov, 1957). A total of 412 observations were made with concentrations of 2.0, 1.654, 1.481, 1.096, and 0.80 mg/m$^3$. The results obtained from the data are listed in Table 1.

It is evident from Table 1 that for the most sensitive persons, the odor threshold of acetone is 1.096 mg/m$^3$, and the subliminal concentration is 0.8 mg/m$^3$.

The odor threshold of acetophenone was determined in 16 persons; a total of 216 determinations were made with 4 acetophenone concentrations. The results of the observations are given in Table 2.

Table 1

Determination of the Threshold of Olfactory Perception of Acetone

| Number of Subjects | Concentration of Acetone, mg/m$^3$ | |
|---|---|---|
| | Minimum Perceptible | Maximum Imperceptible |
| 2 | 2,0 | 1,654 |
| 4 | 1,654 | 1,481 |
| 6 | 1,481 | 1,096 |
| 10 | 1,096 | 0,80 |

Table 2

Determination of the Threshold of Olfactory Perception of Acetophenone

| Number of Subjects | Concentration of Acetophenone, mg/m$^3$ | |
|---|---|---|
| | Minimum Perceptible | Maximum Imperceptible |
| 3 | 0,0251 | 0,0156 |
| 6 | 0,0156 | 0,010 |
| 7 | 0,010 | 0,008 |

The results of our studies are in accord with those of Yu. G. Fel'dman, whose data indicate that the odor threshold of acetone is 1.1 mg/m$^3$, and with those of N. B. Imasheva, whose data show the odor thresholds of acetophenone to be 0.01 mg/m$^3$. After determining the separate odor thresholds, we studied the sensitivity of the olfactory system to mixtures of different concentrations of acetone and acetophenone in the following combinations:

Mixture I - acetone + acetophenone - 1.096 mg/m$^3$ + 0.01 mg/m$^3$.

Mixture II - acetone + acetophenone - 0.711 mg/m$^3$ + 0.0061 mg/m$^3$.

Mixture III - acetone + acetophenone - 0.565 mg/m$^3$ + 0.005 mg/m$^3$.

Mixture IV - acetone + acetophenone - 0.348 mg/m$^3$ + 0.004 mg/m$^3$.

A total of 265 determinations were made on 16 subjects. To obtain comparable results, the concentrations of each ingredient were expressed in fractions of their isolated thresholds. It is known that for a total concentration index equal or close to 1, there is a complete summation of the effect: for an index greater than 1 but smaller than 2, there is a partial summation; and for an index of less than 1, potentiation.

Analysis of the results obtained from the studies showed that the concentration of acetone and acetophenone in the first combination (mixture I) was perceived by all 16 persons. The total concentration index of this mixture ranged from 1.06 to 2.0. The majority of the subjects (12) also identified the odor of the second mixture, in which the sum of the relative concentrations ranged from 1.03 to 1.26. Only the most sensitive persons (5) perceived the third mixture, whose total concentration index was 1.01 (Table 3).

Table 3

Determination of the Threshold of Olfactory Perception of Acetone and Acetophenone in Combination

| | Mixture | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Acetone (mg/m$^3$) | 1,096 | 0,711 | 0,565 | 0,348–0,711 |
| Acetophenone (mg/m$^3$) | 0,010 | 0,0061 | 0,005 | 0,004–0,0061 |
| Sum of fractions of individual thresholds | From 1,06 to 2,0 | From 1,03 To 1,26 | 1,01 | From 0,67 To 0,88 |
| Number of persons perceiving the odor | 16 | 12 | 5 | — |
| Number of persons not perceiving the odor | — | 4 | 11 | 16 |

From Table 3 it is also apparent that the minimum perceptible concentrations in the mixture are 0.565 mg/m$^3$ acetone and 0.005 mg/m$^3$ acetophenone for a sum of relative concentrations equal to 1.01. The mixture is not perceived by its odor if the sum of the relative concentrations is less than 1.

Hence, in this case there is a complete summation of the action of acetone and acetophenone. The summation effect was also found in the case of other, less sensitive persons.

A study of the reflex effect of low concentrations of acetone and acetophenone on the functional state of the central nervous system was made by determining the light sensitivity of the eye under dark adaptation conditions. This test, widely employed at the present time in the practice of sanitary standardization of atmospheric pollutants, makes it possible to record, by means of the reflex physiological reactions, the response to the action of concentrations of toxic substances whose odor is imperceptible but which change the functional state of the cerebral cortex.

A change in the course of the dark adaptation curve during the action of concentrations of noxious substances of imperceptible odor was observed by V. A. Gofmekler (1960), Yu. G. Fel'dman (1960), R. Ubaydullayev (1961), and others.

In a study of the combined action of sulfur dioxide and sulfuric acid aerosol, K. A. Bushtuyeva (1960) observed the phenomenon of physiological summation. She established the fact that an increase in the light sensitivity of the eye as compared with the normal dark adaptation curve during the combined action of these compounds is equal to the sum of the increases during the combined action of these compounds is equal to the sum of the increases during their individual action.

The phenomenon of partial summation was noted by B. K. Baykov (1963) in a study of the action of carbon disulfide in combination with hydrogen sulfide.

Our observations were made on three subjects, 24 to 27 years of age, according to a standard procedure. Persons whose odor threshold was the most sensitive participated in the experiment. A total of 42 tests were conducted. The following mixtures were studied:

1. Acetone + acetophenone - 0.283 mg/m$^3$ + 0.005 mg/m$^3$.
2. Acetone + acetophenone - 0.223 mg/m$^3$ + 0.0037 mg/m$^3$.

The threshold of the reflex effect of acetone on the light sensitivity of the eye for the most sensitive persons is 0.55 mg/m$^3$ according to the data of Yu. G. Fel'dman, and 0.01 mg/m$^3$ according to those of I. B. Imasheva. The total concentration index of the first mixture is 1.01, and of the second, 0.77.

Anaylsis of the results obtained showed that statistically significant changes of the light sensitivity take place only during the action of the first mixture, when the sum of the fractions of the thresholds is equal to 1.01.

In one subject, an increase in light sensitivity was noted after the inhalation of the experimental mixture; in the second a decrease was observed, and the third subject responded to the action of acetone and acetophenone in the first combination first by an increase in light sensitivity (in the 20th minute), and then by its sharp decrease in the 25th minute with a return to the initial level at the end of the experiment. The light sensitivity in the 20th minute of adaptation for all the subjects is given in Table 4.

A mixture of lower concentrations of acetone and acetophenone for a total concentration index of 0.77 was found to be inactive and failed to cause any significant changes in light sensitivity as compared with pure air.

Hence, a complete summation of the action of the compounds studied is also observed in the adaptometric tests.

Table 4

Data of Adaptometric Tests

| Subject | Concentration, mg/m3<br>Acetone + Acetophenone | Light Sensitivity in Relative Units in 20th Minute of Adaptation |
|---|---|---|
| S. R. | Pure Air | 81 475 |
| | 0,283 + 0,005 | 103 050 (b)* |
| | 0,223 + 0,0037 | 82 133 (o) |
| S. L. | Pure Air | 83 225 |
| | 0,283 + 0,005 | 111 000 (b) |
| | 0,223 + 0,0037 | 82 133 (o) |
| Ya. T. | Pure Air | 95 550 |
| | 0,283 + 0,005 | 74 175 (c) |
| | 0,223 + 0,0037 | 90 033 (o) |

* Degree of significance: b - 99%; o - not significant.

We also studied the effect of subliminal concentrations of acetone in combination with acetophenone on the electrical activity of the brain by a quantitative analysis, i.e., the reflex response of a burst of the alpha rhythm of the human brain (A. D. Semenenko, 1964). An analogous procedure was described in the articles of U. G. Pogosyan and Yu. S. Korneyev.

The study was conducted on three subjects on a Galileo polyphysiograph; a total of 62 observations were made. The biocurrents were recorded from the temporal and frontal lobes of both hemispheres and their combinations, and also from the central-parietal part of the left hemisphere. Data obtained from the temporal lobes, which were the most sensitive, were subjected to a graphic statistical analysis. The following mixtures were studied:

1. Acetone + acetophenone - 0.22 mg/m$^3$ + 0.0035 mg/m$^3$.
2. Acetone + acetophenone - 0.18 mg/m$^3$ + 0.0027 mg/m$^3$.

In these studies we also used separate thresholds of action of acetone (0.44 mg/m$^3$) and acetophenone (0.007 mg/m$^3$), established by Yu. G. Fel'dman and N. B. Imasheva. The sum of the relative concentrations (in fractions of threshold values) of the first mixture was equal to 1, and that of the second mixture, to 0.78. The effect of each mixture was studied no fewer than 4 times and was regularly alternated with the supply of pure air.

In our experiments, the first mixture of acetone and acetophenone caused an increase in the energy of the brain potentials in two subjects, whereas in the third, a decrease in the energy of the potentials was noted. At the same time, changes were obtained which were characterized by a statistically significant difference, compared with the control, in the 3rd and 4th minutes of inhalation of the experimental mixture. The quantitative data obtained during inhalation of the second mixture did not differ appreciably from the data obtained during inhalation of pure air.

Thus, the results of studies based on the most sensitive test also confirm the phenomenon of complete summation of subliminal stimuli.

Expressing the data obtained from electroencephalography in fractions of the existing maximum permissible concentrations of each component, we see that the minimum active mixture amounts to 1.79:

$$\frac{0.22 \text{ mg/m}^3}{0.35 \text{ mg/m}^3} + \frac{0.0035 \text{ mg/m}^3}{0.003 \text{ mg/m}^3} = 0.63 + 1.16 = 1.79,$$

and the maximum inactive mixture amounts to 1.41:

$$\frac{0.18 \text{ mg/m}^3}{0.35 \text{ mg/m}^3} + \frac{0.0027 \text{ mg/m}^3}{0.003 \text{ mg/m}^3} = 0.51 + 0.9 = 1.41.$$

On the basis of these data one can postulate that the combined presence of acetone and acetophenone in atmospheric air is permissible if the sum of fractions of these concentrations of their existing maximum permissible values for isolated action does not exceed 1.5.

In order to study the resorptive action of acetone in combination with acetophenone, a chronic inhalational exposure of white male rats was carried out in the course of 84 days. The tests were conducted on 45 rats with an initial weight of 80-100 g, divided into three groups of 15 animals each.

The animals were exposed to mixtures of the indicated compounds in the following average concentrations:

Group I - acetone + acetophenone 1.855 mg/m$^3$ + 0.168 mg/m$^3$.

Group II - acetone + acetophenone 0.192 mg/m$^3$ + 0.00197 mg/m$^3$.

Group III was the control.

Expressing the concentrations of the compounds in fractions of their maximum permissible values for isolated action, we find that the sum of the fractions of the first mixture is equal to 10.9, and that of the second, to 1.2.

Conversion of the acetone and acetophenone concentrations of the second mixture to the threshold concentrations for their isolated action based on the most sensitive test (electroencephalography) shows that the sum of their fractions is equal to 0.71. Hence, animals of group II were exposed to acetone and acetophenone concentrations indifferent for man, whereas animals of group I were exposed to concentrations of these compounds which were 10 times as high.

The action of the mixtures of these compounds was evaluated by observing the general state and weight of the animals; studying the motor chronaxy of antagonist muscles; determining the cholinesterase activity, dynamics of excretion of coproporphyrin, neutral 17-ketosteroids and vitamin C with the urine, and determining the content of the absolute number of eosinophils in peripheral blood.

During the exposure, rats of all the groups were active and gained weight regularly.

In studying the functional state of the central nervous system in the experimental animals during exposure to low concentrations of acetone and acetophenone, we used the method of determination of the motor chronaxy of antagonist muscles. The literature contains indications of a direct relationship between the level of motor chronaxy and the functional state of the central nervous system, which by virtue of subordinational relationships regulates the activity of peripheral nerve apparatus as well as that of lower nerve centers (Yu. M. Uflyand, 1941; A. N. Magnitskiy, 1948).

A manifestation of the subordinational influences of the brain is the constancy of the ratio of the chronaxy of extensors and flexors, whose numerical expression normally amounts to 1.5:1, 2:1, and 2.5:1.

The motor chronaxy was measured in 5 rats of each group three times a month with an ISE-01 electronic pulse stimulator.

Starting with the 4th week of exposure, statistically significant changes in the normal ratio of the chronaxy of extensors and flexors in rats of group I took place chiefly as a result of a prolongation of the chronaxy of flexors. Starting with the 6th week of exposure, a reverse ratio of the chronaxies took place, following which the curves were at approximately the same level and returned to the limits of the physiological norm only during the recovery period.

In rats of group II, no such disturbances of the normal ratio of chronaxy were observed over the course of the entire exposure, and there were no reliable changes as compared with the control.

The changes which we detected in the normal ratio of chronaxies may be regarded as a manifestation of inhibitory processes in the central nervous system which encompass the complex system of cortical and subcortical subordination centers.

The presence of inhibitory processes in the central nervous system of the experimental animals during the action of acetone in combination with acetophenone was confirmed by a depression of the activity of whole blood cholinesterase of the animals.

According to modern interpretations, the transfer of stimulation from a nerve to a muscle is accomplished with the participation of the system acetylcholine - cholinesterase. Here acetylcholine plays the part of a mediator. An excessive accumulation of acetylcholine in the organism causes a decrease in the lability of the nervous processes and the development of a state of inhibition (I. N. Volkova, 1954; D. Ye. Al'pern, 1963).

A decrease in the activity of the enzyme was noted by several authors during a prolonged action of various chemical agents (N. B. Imasheva, 1963; V. A. Chizhikov, 1964; A. V. Mnatsakanyan, 1964, and others).

The cholinesterase activity was determined by the method of J. Fleisher and E. P. Pope (1954). The method is based on a change in the intensity of the color of the solution as a function of the amount of acetic acid formed by the enzymatic cleavage of acetylcholine chloride. The color intensity is determined on an FEK-M instrument.

The cholinesterase activity was determined in 5 rats of each group twice a month; the blood was taken from the tail vein by incising the blood vessel.

It is evident from Fig. 1 that as early as the end of the first month of exposure, the rats of group I showed a considerable decrease in the activity of the enzyme which lasted until the end of the second month. At the end of exposure, the cholinesterase activity became somewhat normal in animals of this group, but remained as before at a lower level than that of the control group. The observed changes in cholinesterase activity are characterized by a statistically significant difference (95-99%).

As far as the cholinesterase activity of animals of group II is concerned, we did not observe any significant changes over the entire course of the exposure.

We estimated the porphyrin metabolism of the experimental animals from the dynamics of the excretion of porphyrin with the urine. The literature contains indications of a direct connection between the porphyrin metabolism and serious disturbances in the central nervous system (A. M. Charnyy and S. E. Krasovitskaya, 1951). A. M. Charnyy postulated that the porphyrins may participate in oxidation-reduction processes in areas of the brain where the cytochrome and cytochrome oxidase are lacking.

The relationship between the excretion of porphyrins from the organism and the activity of bone marrow erythropoesis was pointed out by Watson (1941). In sanitary studies, the porphyrin metabolism was first investigated by M. I. Gusev (1960). During the exposure of rabbits to lead oxide in a concentration of 10 $\mu g/m^3$ for 6 months, the author observed that the amount of coproporphyrin in the urine in this group of animals increased twofold as compared with the control. A disturbance of the porphyrin metabolism was also noted by other authors who studied low concentrations of atmospheric pollutants in chronic experiments (G. I. Solomin, 1962; D. G. Odoshashvili, 1962; V. A. Chizhikov, 1964, and others).

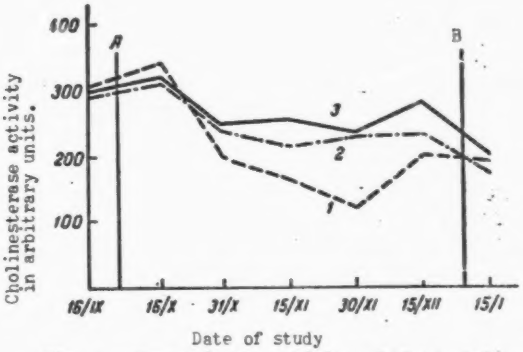

Fig. 1. Change in the activity of blood cholinesterase in rats of different groups.
1 - group I - acetone + acetophenone with total index of 10.9; 2 - group II - acetone + acetophenone with total index of 1.2; 3 - group III - control; AB - period of exposure.

In our studies, we used this test with a quantitative determination of coproporphyrin on an SF-4 spectrophotometer in the ultraviolet region of the spectrum (M. I. Gusev and Yu. I. Smirnov, 1960). The daily urine was collected in special receivers every 15 days from 5 rats of each group. A total of 33 determinations were made, including 9 before the exposure (background), 18 during the exposure, and 6 during the recovery period. Analysis of the data obtained showed that the combined action of acetone and acetophenone in concentrations of 1.855 and 0.0168 mg/m$^3$ respectively caused a definite shift in the porphyrin metabolism of animals of group I.

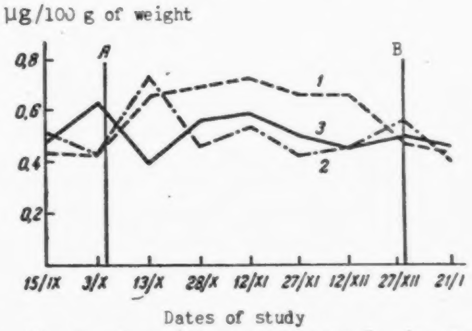

Fig. 2. Excretion of coproporphyrin with the urine in rats of different groups through the effects of acetone and acetophenone.
Notation same as in Fig. 1.

It is evident from Fig. 2 that starting with the 4th week of exposure, the rats of this group showed an increased excretion of coproporphyrin. During the exposure, its content in the urine increased, and during the second period reached a statistically significant difference as compared with the control group (degree of significance, 95%). In group II, no significant differences from the control were observed.

It is known that the activity of the anterior hypophyseal lobe - adrenal cortex hormonal system is controlled by the central nervous system via the hypothalamus.

It was found from literature data that the action of various chemical compounds in high concentrations causes a nonspecific reaction in the anterior lobe - adrenal cortex system; a manifestation of this reaction is a decrease in the absolute quantity of eosinophils in the blood and an increase in the content of neutral 17-ketosteroids in the urine (Ye. I. Spynu, 1962; L. E. Gorn, 1963).

We attempted to detect the presence of nonspecific reactions in the hormonal system in response to the combined action of low concentrations of acetone and acetophenone.

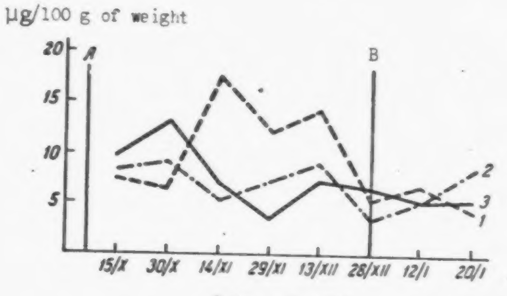

Fig. 3. Content of neutral 17-ketosteroids in the urine of rats of different groups. Notation same as in Fig. 1.

The determination of 17-ketosteroids was made by using the procedure of O. M. Uvarovskaya (1956) followed by colorimetry on an FEK-M instrument with a green filter. The studies were made twice a month, and a total of 24 determinations were performed. A graphical representation of the results obtained is given in Fig. 3, which shows that during the first few days of exposure, the amount of 17-ketosteroids in the urine of animals of group I decreased slightly as compared with the control group. However, starting with the 40th day of exposure, a sharp increase in the content of 17-ketosteroids was noted in rats of group I. Whereas in rats of the control group the amount of 17-ketosteroids during that period was 6.85 µg per 100 g of weight, in rats of group I it was 17.41. The increased level of 17-ketosteroids was preserved until the 70th day of exposure, and at the end of exposure approached the values of the control. The changes obtained were statistically significant.

The fluctuations of 17-ketosteroids observed in the urine of group II of animals did not differ significantly from the control. The eosinophils of the blood were counted by using S. M. Bakman's method (1958). The blood was taken from 5 rats of each group twice a month at a fixed time, and a total of 99 analyses were performed.

In animals of group I after 10 days of exposure, the number of eosinophils increased somewhat, probably as a result of the initial response of the animal organism to the action of acetone and acetophenone (Fig. 4). Subsequently, a sharp decrease in the number of eosinophils was observed which lasted with some fluctuations until the end of exposure. Here the difference in the decrease of the concentration of eosinophils as compared with the control group reached significant values (95%). The number of eosinophils in rats of group II was close to that of the control, although on the 55th day of exposure an increase was observed, but it did not reach a statistically significant difference. An eosinopenic response was observed by A. Ye. Kulakov (1965), who studied the effect of low concentrations of hexamethylenediamine on the organism of animals in a chronic experiment.

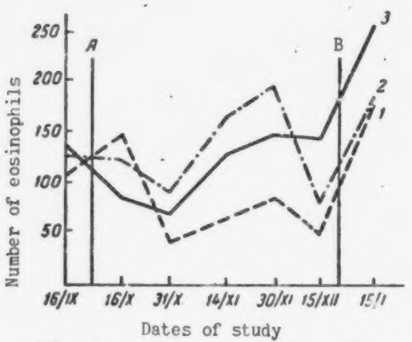

Fig. 4. Absolute number of eosinophils in the blood of rats exposed to the combined action of acetone and acetophenone in low concentrations.
Notation same as in Fig. 1.

Hence, the investigations performed indicate a high sensitivity of our tests and permit the observation of a nonspecific response of the organism to the adverse effect of atmospheric pollutants.

A study of the dynamics of excretion of vitamin C with the urine in white rats over the entire course of exposure failed to reveal any disturbances in the C vitamin balance of the experimental animals. The content of the vitamin in the urine of animals subjected to the exposure did not differ appreciably from that of the control group.

Table 5

Single Concentrations of Acetone in Atmospheric Air Around a Plant Producing Phenol and Acetone

| Distance From Source of Discharges, m | Number of Collected Samples | Number of Samples Below The Sensitivity of the Method | Concentration, mg/m$^3$ | |
|---|---|---|---|---|
| | | | Maximum | Average |
| 100 | 14 | — | 2,19 | 1,41 |
| 300 | 13 | — | 0,714 | 0,415 |
| 500 | 24 | 24 | — | — |

On the basis of the completed studies, we propose a mean daily maximum permissible concentration (in fractions of the existing maximum permissible concentrations) of acetone and acetophenone jointly present in atmospheric air at a level of 1.2. We are aware of the fact, however, that a wide range of unstudied values separates the active (10.9) and inactive (1.2) total concentration of acetone and acetophenone. The Section on Sanitary Protection of Atmospheric Air deemed it possible to approve the mean daily concentration of these compounds (in fractions of their maximum permissible concentrations) at the level of the highest single concentration (1.5).

In order to determine the single concentrations of acetone and acetophenone in atmospheric air around a plant producing synthetic phenol and acetone, we collected 131 samples at distances from 100 to 1000 m from the plant.

As is evident from Table 5, at a distance of 100-300 m from the plant, atmospheric air is considerably polluted with acetone. Among the 27 samples collected at this distance, concentrations substantially exceeding the maximum permissible value (0.35 mg/m$^3$) were observed. Only at a distance of 500 m from the plant was no acetone found in any of the 24 samples.

Table 6

Single Concentrations of Acetophenone in Atmospheric Air Around a Plant Producing Phenol and Acetone

| Distance From Source of Discharges, m | Number of Collected Samples | Number of Samples Below The Sensitivity of the Method | Concentration, mg/m$^3$ | |
|---|---|---|---|---|
| | | | Maximum | Average |
| 100 | 11 | — | 0,877 | 0,258 |
| 300 | 17 | — | 0,097 | 0,057 |
| 500 | 26 | 9 | 0,028 | 0,007 |
| 1 000 | 25 | 25 | — | — |

It is evident from Table 6 that acetophenone is found in substantial concentrations at a distance of 100-500 m from the plant, where its highest concentrations exceed the maximum permissible value (0.003 mg/m$^3$). At a distance of 500 m, the maximum permissible concentration is exceeded in 46% of the samples. Only at a distance of 1000 m from the plant was no acetophenone found in any of the collected samples. Consequently, the sanitary protective zone for this plant should be no smaller than 1000 m.

## Conclusions

1. A determination of the thresholds of olfactory perception of acetone and acetophenone acting individually made it possible to establish that the minimum perceptible concentration of acetone for the most sensitive persons is 1.096 mg/m$^3$, and that of acetophenone, 0.01 mg/m$^3$.

2. When acetone and acetophenone are acting in combination, their odors exhibit a complete summation.

3. The reflex effect of acetone in combination with acetophenone on the light sensitivity of the eye and electrical activity of the brain is characterized by an action expressed in a complete summation.

4. Chronic round-the-clock exposure of white rats to a mixture of acetone and acetophenone for a total concentration index (in fractions of the maximum permissible values) equal to 10.9 causes definite functional shifts in the organism of the experimental animals, manifested in a decrease of the subordinational influence of the brain on the level of the motor chronaxy of antagonist muscles, a depression of the activity of blood cholinesterase, an increase in the excretion of coproporphyrin with the urine, and also the presence of a nonspecific response of the anterior hypophyseal lobe - adrenal cortex system, which we evaluated from the increase in the content of 17-ketosteroids in the urine and a decrease in the absolute number of eosinophils in the blood. An acetone-acetophenone mixture with a total concentration index equal to 1.2 did not cause any significant changes in the animal organism according to the selected tests.

5. In the combined presence of acetone and acetophenone in atmospheric air, their highest single and mean daily concentration expressed in fractions of the maximum permissible values for isolated action should not exceed 1.5.

## LITERATURE CITED

Note: References mentioned in this paper are to be found at the end of this volume in the 1967 bibliography.

# ON THE COMPARATIVE TOXICITY OF BENZENE, TOLUENE, AND XYLENE

## (STUDY OF THE REFLEX EFFECT)

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From Akademiya Meditsinakikh Nauk SSSR. "Biologicheskoe deystvie i gigienicheskoe znachenie atmosfernykh zagryazneniy". Red. V. A. Ryazanova. Vypusk 10, Izdatel'stvo "Meditsina" Moskva, p. 96-108, (1967).

The long-known aromatic hydrocarbons benzene, toluene, and xylene are still attracting the attention of toxicologists and hygienists. This is because these compounds thus far have not lost their industrial importance, possess a marked toxicity and a wide spectrum of action, there is no unified view of the comparative toxicity and mechanism of their action, and the lowest parameters of their effect on the living organism have not been established.

Pure benzene, toluene, and xylene are colorless liquids with a specific aromatic odor, and are poorly soluble in water. The boiling point of benzene is 80.1°C., toluene 110.8°C, and zylene 140.5°C (mixture of isomers). The volatility compared to ether is 3 for benzene, 4.2 for toluene, and 13.5 for zylene. They are good solvents of fats, resins, rubbers, essential oils, dyes, and other organic compounds.

As solvents, the compounds under consideration find extensive applications in various branches of industry. In addition, benzene is widely used in organic syntheses for the production of phenol, nitrobenzene, chlorobenzene, maleic anhydride, and other compounds. Toluene is the starting material in the production of explosives. Xylidenes and phthalic acids are obtained from xylene.

Because of their marked narcotic effect, benzene, toluene, and xylene affect primarily the functions of the nervous system. Thus, G. E. Rozentsvit (1954) emphasizes that functional disturbances of the central nervous system precede morphological changes in the blood. Some investigators even go so far as to consider the changes in other organs as consequences of damage to the nervous and endocrine systems.

A change in the reflex activity of animals acted upon by various concentrations of benzene was observed by R. I. Yaroslavskaya and I. M. Rozovskiy (1952), Yu. V. Novikov (1957), A. I. Korbakova, S. N. Kremneva, N. K. Kulagina, and I. P. Ulanova (1960).

In exposures of mice to xylene in a concentration of 550 mg/m$^3$, A. A. Golubev (1959) noted a lag in the rate of development of conditioned reflexes and a decrease in the regularity of differentiated responses.

Ye. N. Lyublina (1950) determined the minimum concentrations of benzene, toluene, and xylene acting on the central nervous system of rabbits. For a 40 minute exposure, they were 300, 500, 1000 mg/m$^3$ for benzene, 300, 500, and 1000 mg/m$^3$ for toluene, and 100, 200, and 400 mg/m$^3$ for xylene.

Concentrations disturbing the unconditioned reflex activity of rabbits were 1500 for benzene, 1000 for toluene, and 750 mg/m$^3$ for xylene (A. S. Faustov, 1961). The author emphasizes that in contrast to benzene and toluene, xylene causes a depression of the central nervous system.

The comparative evaluation of the toxicity of benzene, toluene, and xylene is made by most authors on the basis of the action of these compounds on the blood and blood-forming organs. From this standpoint, M. L. Mgebrov, N. D. Rosenbaum, Lind, and others consider benzene to be more toxic. Others indicate that chronic poisoning with toluene may be associated with a depression of the activity of the bone marrow, as in the case of benzene. R. G. Leytes, B. I. Martsinkovskiy and L. K. Khotsyanov hold that toluene and xylene act like benzene, but that their action is associated with less pronounced changes in the blood (O. N. Olimpiyeva, V. M. Retnev, and A. P. Rusinova, 1958).

The study and comparative evaluation of minimum concentrations of these compounds on the organism are of interest from the standpoint of both toxicology and sanitary protection of atmospheric air.

There are scant literature data on the pollution of atmospheric air with vapors of benzene, toluene and xylene. The sources of atmospheric pollution may be not only major coking plants and petroleum refineries (A. A. Itskovich and V. A. Vinogradova, 1956; M. L. Krasovitskaya and T. S. Zaporozhets, 1961), but also small enterprises and printing houses which use these compounds as solvents (Yu. V. Novikov, 1956).

With their distinct, specific odor, benzene, toluene, and xylene are able to act on the receptors of the upper respiratory tract and nasal cavities and cause a series of reflex responses involving the nervous system and its highest stage, the cerebral cortex. "The odors and the reflexes that they cause are by no means indifferent for the organism. Since they are reflected in the cortex, they can produce time relationships and involve the most diverse processes in their action" (S. V. Anichkov, 1952).

In recent years, a number of investigations have shown that chemical compounds in concentrations coinciding with the threshold of olfactory perception or slightly above it are able to cause such reflex responses as a change in the vascular tone, rhythm and depth of respiratory movements, optimal chronaxy, galvanic skin reflex, and the light sensitivity of the eye (K. A. Bushtuyeva, 1960; M. K. Borisova, 1960; M. T. Takhirov, 1960, and others).

On this basis, we undertook a study of the thresholds of olfactory perception in order to obtain a comparative evaluation of the effect of these compounds. The thresholds of olfactory perception of benzene, toluene, and xylene had already been studied. However, the studies were conducted by different experimenters using different procedures, and the threshold values for the same substance differed considerably. Thus, N. V. Lazarev (1963) gave the following thresholds of olfactory perception: benzene 5 mg/m$^3$, toluene 2 mg/m$^3$, and xylene 0.8 mg/m$^3$. According to the data of Yu. V. Novikov (1956), the threshold of olfactory perception of benzene is 3 mg/m$^3$. Ch'en Yun-t'ai established the threshold of olfactory perception of ortho-xylene at the level of 0.73 mg/m$^3$ (1963).

We made a determination of the thresholds of olfactory perception according to a procedure recommended by the Committee on Sanitary Protection of Atmospheric Air (V. A. Ryazanov, K. A. Bushtuyeva, and Yu. V. Novikov, 1957).

The concentrations of benzene, toluene, and xylene were determined by using M. V. Alekseyeva's procedure (1964). It is based on nitrating these compounds, then extracting the nitro compounds with butanol. When a base is added to the extract, the solution acquires a certain color (purple for benzene, orange for toluene, and greenish-blue for meta-xylene). The color of the solutions was measured colorimetrically. The determination was made with the aid of photoelectrocolorimeter (FEKN-57) and calibration curve. The sensitivity of the method was 0.5 μg for benzene, 1 μg for toluene, and 2 μg for xylene in a volume of 2 ml. The samples were collected before and after each observation, and the fluctuations of the concentrations were insignificant.

Table 1

Results of Determination of the Olfactory Perception Threshold for Benzene Vapors

| Number of Subjects | Concentration of Benzene, mg/m$^3$ | |
|---|---|---|
| | Minimum Perceptible | Maximum Imperceptible |
| 8 | >4,0 | — |
| 3 | 3,4 | 3,2 |
| 2 | 3,2 | 3,0 |
| 5 | 2,8 | 2,5 |

The threshold of olfactory perception of benzene was determined on 18 subjects. A total of 7 concentrations were studied, and 518 tests were conducted. The results are shown in Table 1.

The first benzene concentration studied, 4 mg/m$^3$, was imperceptible for 8 persons out of 18. The minimum perceptible concentration for the 5 most sensitive persons was 2.8 mg/m$^3$, and the maximum imperceptible concentration was 2.5 mg/m$^3$. Thus, the results of our studies confirm the data on the olfactory perception threshold obtained by Yu. V. Novikov in 1956 (3 mg/m$^3$).

Table 2

Results of Determination of the Olfactory Perception Threshold for Toluene Vapors

| Number of Subjects | Concentration of Toluene, mg/m$^3$ | |
|---|---|---|
| | Minimum Perceptible | Maximum Imperceptible |
| 5 | >3,2 | — |
| 6 | 3,2 | 2,8 |
| 4 | 2,8 | 2,3 |
| 3 | 2,3 | 2,0 |
| 3 | 2,0 | 1,8 |
| 3 | 1,8 | 1,5 |
| 6 | 1,5 | 1,27 |

The threshold of olfactory perception of toluene was studied on 30 subjects. A total of 7 concentrations were studied, and 744 observations were made, the results of which are presented in Table 2.

Of 30 persons, 5 did not perceive the toluene concentration studied - 3.2 mg/m$^3$; the 1.5 mg/m$^3$ concentration was found to be the minimum perceptible value for the most sensitive 6 subjects. The maximum imperceptible concentration for the same subjects was 1.27 mg/m$^3$. Thus, the threshold of olfactory perception of toluene, 1.5 mg/m$^3$, was found to be slightly below the threshold given by N. V. Lazarev (2 mg/m$^3$).

The determination of the threshold of olfactory perception of meta-xylene was performed on 18 persons. Six concentrations were studied, and a total of 431 observations were made. The results of the experiment are shown in Table 3. It is evident from the latter that the minimum perceptible concentration of meta-xylene for the foremost sensitive subject is 0.6 mg/m$^3$, and the maximum imperceptible concentration is 0.41 mg/m$^3$. Hence, the threshold of olfactory perception of meta-xylene (0.6 mg/m$^3$) practically coincides with the threshold for the ortho and meta isomers of xylene (0.73 mg/m$^3$) obtained by Ch'en Yun-t'ai.

Thus, a lowering of the thresholds of olfactory perception was observed from benzene to xylene. The increase in the number of methyl groups in the benzene ring probably causes an intensification of the odor.

The next stage of the comparison of the toxicity of benzene, toluene, and xylene were studies of the effect of their minimum concentrations on the electrical activity of the cerebral cortex.

The rhythm of the electric potentials of the brain constitutes an electric manifestation of the processes occurring in different regions of the cortex of the cerebral hemispheres and other formations of the central nervous system.

Table 3

Results of Determination of the Threshold of Olfactory Perception of Xylene Vapors.

| Number of Subjects | Concentration of Xylene, mg/m$^3$ | |
|---|---|---|
| | Minimum Perceptible | Maximum Imperceptible |
| 2 | 1,9 | 1,4 |
| 3 | 1,4 | 1,0 |
| 5 | 1,0 | 0,85 |
| 4 | 0,85 | 0,6 |
| 4 | 0,6 | 0,41 |

K. A. Bushtuyeva, Ye. F. Polezhayev, and A. D. Semenenko (1960) were the first to apply the method of functional electroencephalography to the study of the reflex effect of minimum concentrations of chemical agents. They found that concentrations which are below the threshold of olfactory perception and which as such do not produce any visible effect, can affect the electrical activity of the cerebral cortex.

At the present time, several modifications of functional electroencephalography are used to study the effect of minimum concentrations of chemical substances on the central nervous system. In order to study the effect of odor subthreshold concentrations of benzene, toluene, and xylene, we chose the method of quantitative analysis of the reflex response of the alpha rhythm flare-up (A. D. Semenenko, 1963), which in our view better reflects the dynamics of the processes occurring in the cerebral cortex. The method is based on the phenomenon of reinforcement of the electric potentials by various rhythmic stimuli whose frequency corresponds to the intrinsic rhythm of the brain. Stimulation in rhythm with the brain potentials (trigger stimulation) was proposed in 1949 by Walter and Shipton. N. P. Bekhtereva and V. V. Usov (1960) noted that trigger stimulation may considerably reinforce the alpha rhythm and produces a greater effectiveness of the functional load.

In our studies, the stimulation was carried out by using a rhythmic light whose intensity was changed every 5 seconds in order to increase the functional load on the central nervous system. The study of the effect of low concentrations of the compounds studied on the electrical activity of the cerebral cortex was started after developing a synchronous and well-defined alpha rhythm in the subject. The experiments were performed in a specially equipped, shielded, and faintly illuminated chamber. The subject was in a semireclining posture in a soft, easy chair. In front of his face was placed a cylinder through which pure air was supplied at a rate of 30 l/min, and at the desired moment, this air was combined with vapors of the investigated compound in a given concentration. The device for adjusting the supply of vapors, the electroencephalograph, and the instruments controlling the stimulators were located outside the chamber.

The study of the effect of each concentration consisted of 10 observations representing the alternation of the recording of biocurrents during inhalation of the compound in a given concentration (five observations) and during inhalation of pure air (five observations). A single observation consisted of 18 one-minute cycles, each of which included the application of a sound stimulus - 10 seconds, waiting for the light - 7 seconds, application of photic stimulus - 18 seconds, and 25 seconds of active limbering up, done by the subject in order to produce a stable rhythmic stereotype and to maintain the general tone.

The eighteen minutes of the experiment consisted of three minutes of training, followed by three minutes forming the background for the given observation, with the remaining 12 minutes devoted to the experiment proper. During the administration of the compound, the "gas" was supplied for 6 minutes, and the recovery period lasted 6 minutes. The gas-air mixture was supplied intermittently, beginning immediately before the sound was turned on and ending when the photic stimulus was turned off.

Analysis of the recording of the biocurrents was based on the integrated energy of the reinforced rhythm. The results of the tests were treated statistically and involved the determination of the confidence factor of the changes obtained.

The reflex effect of benzene in concentrations of imperceptible odor on the electrical activity of the cerebral cortex was studied on 5 subjects most sensitive to the odor threshold. The biopotentials were recorded on a 16-channel electroencephalograph of the Galileo Co. The biocurrents were taken off the temporal, occipital, and frontal regions and their combinations, bipolarly. A benzene concentration of 2 mg/m$^3$ caused a reinforcement of the electric potentials from the left temporo-occipital regions of the brain. The dynamics of these changes can be followed from the data of Table 4.

It is evident from Table 4 that statistically significant changes of electric potentials appear in the 3rd-4th minute of supply of the gas-air mixture. The reliability of these changes increases by the 5th-6th minute of supply of the gas-air mixture and disappears by the 5th-6th minute of the recovery period. No significant changes were detected in the statistical treatment of recordings from other regions of the brain. Inhalation of benzene in a concentration of 1.5 mg/m$^3$ by the subjects did not cause any significant changes in the biopotentials of the brain.

Table 4

Significance of Changes in the Electric Potentials of the Brain During Inhalation of Benzene in a Concentration of 2 mg/m$^3$

| Subject | Gas-Air Mixture | | | Recovery Period | | |
|---|---|---|---|---|---|---|
| | Minutes | | | Minutes | | |
| | 1–2 | 3–4 | 5–6 | 1–2 | 3–4 | 5–6 |
| A. S. | o | b | c | a | a | o |
| K. S. | o | a | c | o | o | o |
| M. Z. | o | a | b | o | c | o |
| N. V. | o | c | o | b | b | o |
| S. L. | o | b | c | c | c | o |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%; o - insignificant.

The change in the electric potentials of the brain in subject N. during inhalation of different benzene concentrations is shown in Fig. 1.

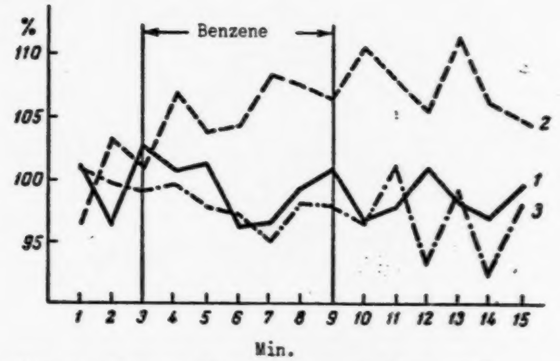

Fig. 1. Change in the electric potentials of the brain of subject N. during inhalation of various benzene concentrations.

1 - pure air; 2 - concentration 2 mg/m$^3$; 3 - concentration 1.5 mg/m$^3$.

The reflex effect of odor subthreshold concentrations of toluene on the electrical activity of the cerebral cortex was studied on subjects with the most sensitive odor threshold. The biocurrents were recorded from the fronto-central and temporo-occipital regions. In a concentration of 1 mg/m$^3$, toluene caused a distinct, statistically significant reinforcement of the electric potentials from the left fronto-occipital regions of the brain in all the subjects. The reliability of these changes increased by the 5th-6th minute of supply of the gas-air mixture and remained until the end of the recovery period (Table 5).

In the remaining recordings, no changes in the electric potentials were found during inhalation of 1 mg/m$^3$ of toluene and none were detected in any recordings during inhalation of 0.6 mg/m$^3$.

Table 5

Reliability of Changes in the Electric Potentials of the Brain During Inhalation of Toluene in a Concentration of 1 mg/m$^3$.

| Subject | Gas-Air Mixture | | | Recovery Period | | |
|---|---|---|---|---|---|---|
| | Minutes | | | Minutes | | |
| | 1—2 | 3—4 | 5—6 | 1—2 | 3—4 | 5—6 |
| V. S. | b | o | o | c | a | o |
| N. V. | b | o | c | b | c | c |
| P. N. | o | c | b | c | b | a |
| S. L. | a | a | a | a | a | c |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%
o - insignificant.

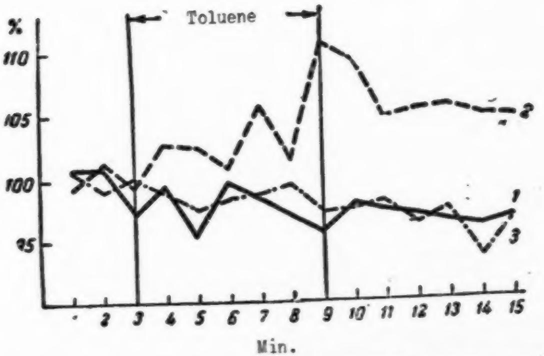

Fig. 2. Change in the electric potentials of the brain of subject N. during inhalation of different concentrations of toluene.

1 - pure air; 2 - concentration 1 mg/m$^3$; 3 - 0.6 mg/m$^3$

Data on the change in the electric potentials of the brain of subject N. during inhalation of different concentrations of toluene are presented in Fig. 2. The reflex effect of xylene was also studied on 4 subjects with the most sensitive odor threshold. The biocurrents were recorded from the fronto-occipital region. The first xylene concentration studied, 0.32 mg/m$^3$, caused a distinct, statistically significant decrease of the electric potentials from both fronto-occipital regions of the brain in all the subjects. These changes were more pronounced in the left fronto-occipital region. The degree of significance increased by the 5th-6th minute of supply of the gas-air mixture and, without decreasing, was preserved until the end of the recovery period (Table 6). A xylene concentration of 0.21 mg/m$^3$ caused no changes whatsoever. The change in the electric potentials of the brain of subject N. during inhalation of different xylene concentrations is shown in Fig. 3.

Table 6

Significance of Changes in the Electric Potentials of the Brain During Inhalation of Xylene in a Concentration of 0.32 mg/m$^3$.

| Subject | Gas-Air Mixture | | | Recovery Period | | |
|---|---|---|---|---|---|---|
| | Minutes | | | Minutes | | |
| | 1–2 | 3–4 | 5–6 | 1–2 | 3–4 | 5–6 |
| A. K. | o | a | o | b | a | o |
| N. V. | o | b | c | b | c | c |
| P. N. | a | o | b | c | b | c |
| S. L. | b | a | a | b | b | b |

Note. Degree of significance: a - 95%; b - 99%; c - 99.9%; o - insignificant.

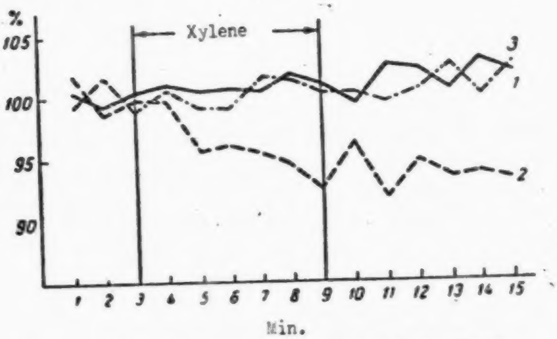

Fig. 3. Change in the electric potentials of the brain of subject N. during inhalation of different concentrations of xylene.

1 - pure air; 2 - concentration 0.32 mg/m$^3$; 3 - concentration 0.21 mg/m$^3$

Table 7

Results of Study of the Threshold of Olfactory Perception and Reflex Effect of Microconcentrations of Benzene, Toluene, and Xylene on the Electrical Activity of the Brain.

| Substance | Olfactory Perception | | Reflex Effect on Biocurrents of Brain | |
|---|---|---|---|---|
| | Concentration, $mg/m^3$ | | | |
| | Threshold | Subthreshold | Threshold | Subthreshold |
| Benzene | 2,8 | 2,5 | 2,0 | 1,5 |
| Toluene | 1,5 | 1,27 | 1,0 | 0,6 |
| Xylene | 0,6 | 0,41 | 0,32 | 0,21 |

Comparing the results of the studies (Table 7) and evaluating the character of the action of microconcentrations of benzene, toluene and xylene on the electrical activity of the cerebral cortex, one can reach the following conclusions:

1. The thresholds of olfactory perception are lowered as the number of methyl groups in the benzene ring increases.

2. The magnitude of the threshold concentrations from the standpoint of the effect on the electrical activity of the brain decreases from benzene to xylene in proportion to the decrease of the thresholds of olfactory perception.

3. Benzene and toluene reinforce the electric potentials; xylene has the opposite effect, causing a marked depression of the electrical activity of the cerebral cortex. The restoration of electrical activity of the brain during the action of toluene and xylene takes place more slowly.

4. Concentrations of 1.5 $mg/m^3$ benzene, 0.6 $mg/m^3$ toluene and 0.2 $mg/m^3$ xylene are the subthreshold ones for the effect on the electrical activity of the brain and their odor is imperceptible; we therefore recommend them as the highest single maximum permissible concentrations for atmospheric air. Our proposal was examined and approved by the section on sanitary protection of atmospheric air of the All-Union Problems Commission.

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Note: References mentioned in this paper are to be found at the end of the volume in the 1967 bibliography.

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